Identification of risk factors associated with aetiology of amyotrophic lateral sclerosis based on systematic review and meta-analysis

MS candidate, Ming-Dong Wang

Supervisors: Daniel Krewski, James Gomes

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Faculty of Medicine
University of Ottawa

Contents

Abstract

To identify the risk factors being associated with aetiology of amyotrophic lateral sclerosis **(**ALS), a series of systematic reviews based on existing observational epidemiological studies identified through searching of bibliographic databases were conducted. Associations between ALS and a number of genetic and environmental risk factors were examined using meta-analysis. Specifically we found that previous exposure to lead, pesticides, solvents, experience of trauma and electric shock were associated with relative increased risks of developing ALS of 86% [odds ratio (OR) =1.86, 95% CI: 1.39-2.48], 57% (OR=1.57,95% CI: 1.19-2.08), 47% (OR=1.47, 95%CI: 1.13-1.80), 64% (OR=1.64; 95%CI: 1.36-1.98), and 2.27% (OR=3.27, 95%CI:1.87-5.73) respectively, compared to their corresponding controls. The presence of intermediate CAG repeat expansion in the *ATXN2* gene was associated with a 4.4 -fold increase in the risk of ALS (OR=4.44, 95%CI: 2.91-6.76). However, the attributable risk associated with each identified risk factor was estimated to be less than 5% of all ALS cases. These results confirm that ALS is a rare multifactorial degenerative condition of motor-neurons.

Executive summary

E.1. Background and Objective

Amyotrophic lateral sclerosis (ALS), the most common subset of motor neuron diseases, (MND) is a rare multifactorial degenerative condition of motor neurons, characterized by rapid irreversible progression. It presents either in a familial (FALS) or sporadic (SALS) form. FALS and SALS account for 5-10% and 90-95% of all ALS cases respectively (1). It starts with bulbar onset in one third and spinal onset in two thirds of all ALS cases (2). The average age at onset is about 60 years. The average age at onset of ALS cases with bulbar onset is greater than that for cases with spinal onset (2). The average survival time from diagnosis is about 2-3 years for ALS cases with bulbar onset and 3-5 years for ALS cases with limb onset (2). The causes of ALS are still largely unknown, although a small proportion (about 10%) of ALS cases are related to monogenic mutations found in most FALS cases, and in some SALS cases (3). The two most commonly mutated monogenic genes among white ALS cases are *SOD1* and *C9orf72* among white ALS patients, accounting for 25% and 35% of FALS cases, respectively, and 2% and 6% of SALS cases, respectively (2-6). In other ethnic groups, the *C9orf72* mutation is rare (6), because the *C9orf72* mutation arose in Scandinavia a few thousand years ago (7,8). Most ALS cases (90% of all ALS cases) are thought to be caused by polygenic variants/polymorphisms, and/or via an interaction between genetic and environmental risk factors (9). Although many genetic and environmental risk factors have been found to be associated with aetiology of SALS cases, no single gene variant or environmental risk factor has been firmly associated with aetiology of SALS in the epidemiological studies conducted to date. Previous observational studies have not been comprehensively reviewed, nor have the data been adequately analysed and synthesized to draw overall, evidence-based conclusions about risk factors for ALS using systematic review strategies. Here, our objective is to identify risk factors associated with the occurrence of ALS using these strategies.

E.2. Methods

Existing systematic reviews and observational studies related to ALS disease development were searched from Medline, Pubmed, Embase, Hugenet, Toxiline through to March of 2013. Related articles were also tracked from Pubmed at least once a week thereafter. A priori

criteria were used to search scientific literature for all risk factors (genetic, environmental, lifestyle) for ALS. All identified articles were screened using DistillerSR software with predesigned screening forms based on inclusion/exclusion criteria (English, epidemiological observational study, systematic review, meta-analysis, ALS, and risk factors). Data from moderate or high quality existing systematic reviews were summarized and updated with new observational studies. If more than 5 observational articles for a related risk factor were found, a meta-analysis would be conducted with RevMan5.1 software. Genetic factors associated with ALS were summarised from an existing ALS associated gene website (10), and from the results of a PubMed search.

E.3. Results

Three environmental chemical risk factors (exposure to lead, pesticides and solvents), physical factors (mechanical injury, electric shock), and genetic factors (intermediate CAG repeat expansion in *ATXN2*) were found to be associated with increased risk for ALS. However, monogenic gene mutations in about two dozen genes have been identified and associated with ALS. Perhaps common lifestyle risk factors, cigarette smoking and alcohol consumption, both of which are able to regulate neurological functions, have not been found to be strongly associated with the development of SALS.

E.3.1. Intermediate CAG repeat expansion in the ATXN2: The odds of intermediate CAG repeat (31-34) expansion in the ***ATXN2*** gene among ALS patients was about 3.9 fold higher than among controls [random effects, OR=3.93(2.49-6.20)]. Interestingly, the OR [3.59 (1.58- 8.17), random effects model] in FALS cases was not statistically significantly higher than the OR [3.01 (1.77- 5.11)] in SALS cases.

E.3.2. Antecedent exposure to lead: The odds of experiencing exposure to lead among ALS patients compared to controls was significantly relatively increased by 86% [random effects, OR=1.86 (1.39, 2.48)]. The attributable risk due to previous exposure to lead was estimated to be less than 5% of total ALS cases.

E.3.3. Pesticides: The odds of previous exposure to pesticides including agricultural chemicals among ALS cases versus their corresponding controls increased by about 57%, with negligible heterogeneity [random effects, OR=1.57 (1.19-2.08)]. The attributable risk

due to previous exposure to pesticides to ALS was estimated to be less than 4% of total ALS cases.

E.3.4. Previous trauma: The odds of previous experience of trauma among ALS patients was over 70% higher than controls, [random effects, OR=1.64(1.36-1.98)], with limited heterogeneity across included studies. The attributable risk due to previous trauma to ALS was estimated to be less than 5% of total ALS cases. Old trauma (defined as trauma that occurred at least 5 years prior to ALS diagnosis) was also significantly associated with ALS [random effects OR=1.40(1.06-1.86)]. Subgroup analyses revealed that previous head trauma was associated with increased risk of ALS [random effects, OR=1.27(1.02-1.57)]. Our meta-analyses from case-control studies revealed that body mass index (BMI) measured prior to ALS onset was associated with a significantly decreased risk of developing ALS [random effect, average difference=-0.24(-0.34 - -0.14)].

E.3.5. Organic solvents: The odds of previous exposure to solvents among ALS patients versus controls was increased by more than 40%, with no significant heterogeneity [random effects OR=1.43(1.10-1.86)] or publication bias (data not shown) across seven included case-control studies.

E.3.6. Previous electric shock: Meta-analysis from all 6 included case-control studies related to the electric shock showed that antecedent electric shock was strongly associated with increased risk of ALS [random effects OR=3.27(1.87-5.73)].

E.3.7. Cigarette Smoking: A mild excess relative risk (RR) for developing ALS in female smokers as compared to non-smokers was identified based on three cohort studies, with no significant heterogeneity [random effects RR(relative risk)=1.34(1.17-1.55)].

E.4. Conclusion

Multiple risk factors are associated with increased risk for ALS. In this systematic review, we identified a number of potential risk factors via meta-analyses of existing observational studies. It is worth noting that these associations are not necessarily causal, regardless of how many case-control studies were included in each meta-analysis. Since

there were few cohort studies available for inclusion in this study, it is not possible to draw an overall conclusion on risk factors for ALS based on data from the cohort studies, except for smoking. The risk factors identified here need to be verified by using well designed cohort epidemiological studies, supplemented by laboratory studies designed to elucidate the biological mechanisms of action by which such factors may lead to the development of ALS.

Chapter1: About the study

1.1. Study Hypothesis: ALS is a rare multifactorial degenerative condition of motor neurons, characterized by rapid irreversible progression. However, to date, no single environmental risk factor has been firmly associated with aetiology of SALS in literature (11). We hypothesize that the primary reason is that the approaches of systematic review and meta-analysis have not been adequately exploited to synthesize the existing scientific evidence for understanding the aetiology of ALS. Therefore, the research question for my MSc thesis in epidemiology will be: What risk factors for the development of SALS can be identified based on existing observational studies, using systematic review and meta-analysis?

1.2. Rational of the thesis: Epidemiological studies have shown that the occurrence of SALS was associated with exposure to multiple environmental factors (11-13) and multiple genetic risk factors (14-16). It is estimated that genetic factors and environmental risk factors played an equal important role in contribution to the total occurrence of ALS cases (17,18). Although numerous potential environmental risk factors for ALS have been investigated in many previous observational studies, and summarized in general reviews, few systematic reviews dealing with risk factors for ALS had been conducted (12,13,19). As a consequence, the association between a given risk factor (particularly for environmental risk factors, and gene variants) and the occurrence of SALS has never been unambiguously established in the context of observational studies of human populations. The reasons for this situation include the followings. (a), since ALS is a rare disorder, observational studies are not common; (b), since ALS is a rare multifactorial condition, the contribution to the total ALS cases by each risk factor is minor, rendering the identification of these factors more difficult. Thus the conflicting results are found common in literature; (c), the approaches of systematic review and meta-analysis have not been adequately exploited to understand the aetiology of ALS, since meta-analysis allows combining multiple studies to increase the statistical power. With the exception of a limited number of generic reviews on this topic, only three comprehensive systematic reviews could be found in literatures (12,13,19). Of these, only one systematic review with a meta-analysis focussing on a single risk factor (smoking) was identified (19). The meta-analysis was not adequately conducted because the cohort and case-control studies

were combined together in this systematic review. In addition, other two systematic reviews with no meta-analyses were published by the same authors: one of these discussed all possible risk factors potentially associated with the occurrence of SALS, and the other focused on occupational exposure (12,13). Therefore, a comprehensive systematic review of the potential risk factors for SALS is needed in order to synthesize previous observational studies and identify potential risk factors associated with the occurrence of ALS.

1.3. Significance of this study to Canadians: The prevalence and incidence of ALS in Canada is comparable to that in other European or North American countries, according to studies in Southern Ontario, Nova Scotia, and Alberta (20); FALS rates in Canada are also comparable to international rates(4). ALS has imposed a heavy social and economic burden on ALS affected families and the health care system in Canada (PHAC, 2007). However, there is a shortage of information about ALS, particularly on possible causes of this severe neurological condition. Canada had no national level ALS registration system until early 2013 (21). The identification of environmental risk factors associated with the occurrence of ALS in Canada will help ALS affected Canadians, their family members, and health care providers identify strategies to mitigate this devastating condition and improve the quality of life among ALS affected individuals.

1.4. Support for this study: The material in this thesis is part of a systematic review of risk factors affecting the onset and progression of 14 neurological conditions conducted under the National Population Health Study of Neurological Disease in Canada sponsored by Public Health Agency of Canada (2009-2013).

1.5. Analytic strategy: This project was conducted within two phases. In the first phase, we searched the scientific literature for existing systematic reviews and meta-analyses related to genetic and environmental risk factors for ALS from multiple bibliographic data bases. In the second phase, relevant observational studies focusing on risk factors for ALS were searched from multiple bibliographic databases through to March 18, 2013. Similar articles were tracked in PubMed at least once a week after that date. The gray literature was searched using Google Scholar. Some relevant articles were hand-searched based on the reference lists of selected articles, and by contacting the article authors. All searched records were screened

with predesigned forms in DistillerSR against predetermined inclusion and exclusion criteria. The selected systematic reviews and meta-analyses were evaluated using AMSTAR scale (22). The systematic reviews or meta-analyses with moderate or above quality (scores>=4) were retained for data extraction, and further updated with results from new observational studies. The selected observational studies were evaluated using modified Downs and Black's criteria (23). The data were extracted from selected articles and, where appropriate, synthesized by meta-analysis. The systematic reviews and meta-analyses of observational studies were prepared according to Moose's system and PRISMA guidelines (24,25). Further details are provided in appendix A.

1.6. Structure of the thesis: This is a manuscript based thesis, written according to the guidelines for thesis preparation from the department of Epidemiology and Community Medicine, University of Ottawa. This thesis is comprised of a chapter providing an overview of the literature in this area, followed by four manuscripts, concluding with a summary and discussion of the main findings.

1.7. Contribution of the authors: All manuscripts were prepared primarily by Ming-Dong Wang under the supervision of Drs. Daniel Krewski and James Gomes. Dr. Krewski also contributed significantly to manuscript preparation, with Dr. Gomes contributing to record screening. Dr. Cashman and Dr. Little reviewed the manuscripts in detail, and contributed significantly to the evaluation and interpretation of the study results.

Chapter 2: Literature review

2.1. Overview: Amyotrophic lateral sclerosis (ALS) is a rare neuron-degenerative condition affecting voluntary muscles progressively (26-28). The typical symptoms/signs in ALS patients are the progressive weakness (and/or atrophy and paralysis) of affected voluntary muscles, with the weakness spreading from the site of onset to other areas until all voluntary muscles affected. The functions of sphincters, the muscles responsible for ocular movement, and the tissues controlled by sensitive nerves (temperature, pain, taste, hearing, sighting, smelling) are usually spared. However, dementia, cognitive impairment, and executive dysfunction were detected in more than 50% of ALS patients (29-31), especially for the patients with mutation in *C9ORF72* gene (32-35). In most of ALS cases, ALS progression is rapid. The average survival time from diagnosis is less than 3 years, often due to the failure of respiratory functions. Notably, a small proportion (10-20%) of ALS cases could survive for greater than 10 years (36), even some rare ALS cases could survive for greater than 50 years (37). Multiple ALS subsets and ALS variants have been described (38), and a new subset of MND has also been proposed (39), indicating the heterogeneity of ALS phenotypes and genotypes (16,27,40,41), even among individuals with same type of gene mutations such as C9ORF72(42,43), *SOD1*(27) and *FUS* mutation(44) .

2.2. Incidence: The crude incidence of ALS across various studies in Europe and North America, especially among whites, is quite stable, falling within a range of 2-3 cases per 100,000 population per year (45,46). The median incidence was 2.08 cases per 100,000 persons per year and the median prevalence is 5.40 cases per 100,000 persons per year, as reported in European countries (47). Although the incidence of ALS in Asia is lower in general population (47), the highest incidence (50-100 fold greater than the global average) ever reported was also seen in Asia (Guam and surrounding islands) during the period of 1950s-1960s (48-53). Populations with Hispanic or Black backgrounds are believed to be

relatively resistant to ALS (45). Since the survival time is short for ALS patients, the mortality of ALS roughly reflects its incidence (54).

Both the incidence and prevalence of ALS is greater in men than in women, with the ratio of occurrence of ALS in men as compared to women being around 1.5 (with a range of 1.1-3.0) (55). However, the male preponderance doesn't occur similarly across all subsets of ALS cases. The male preponderance is associated with onset age, disease types, and may also be associated with its causes (56). The highest male preponderance has been observed in young-adult onset patients with pure upper motor neuron (p-UMN) ALS subset (5.8:1)(57). However, this preponderance was not supported by a subsequent study in Italy (40). A male preponderance in respiratory, flail arm, classic and pure lower motor neuron (PLMN) phenotypes was observed (40). In all other phenotypes [flail leg, pyramidal, and pure upper motor neuron (PUMN)], the incidence rates among men and women are similar (40). In addition, this male preponderance has declined over last decades, suggesting that exposure to environmental risk factors for ALS could explain part of this gender difference (56).

ALS is an age-associated neurodegenerative condition, but not an aging associated condition like Alzheimer's disease (58). Like some types of chronic diseases such as diabetes and multiple sclerosis, the incidence of ALS increases with age, and reaches the peak at around the age of 70 years old, then rapidly declines (46,47). If ALS is an aging-associated disease like Alzheimer's disease, this decline would not happen. Although ALS is a rare neuron degenerative condition, studies found that its contribution to total human death has increased over last few decades, and more so in women than in men (56). Therefore, in light of the challenge of aging population increases worldwide, exploring the causes of ALS becomes imperative.

Incidence in Canada: Up to now, only 5 epidemiological studies of ALS have been conducted in Canada. Three studies were done in Nova Scotia and Newfoundland, one in Southern Ontario, and one in Alberta. These studies have been synthesized by Wolfson and colleagues (20). The incidence of ALS in Canada was estimated to be 2.4 cases per 100,000 persons per year, similar to that in European countries and the US (47). According to this estimate, then about 800 new ALS cases are diagnosed each year in Canada, and about 2000

Canadians now live with ALS. The point prevalence of FALS in Alberta was approximately 7.38 per 1,000,000 (95% CI 2.42-8.18) (4). Recently an ALS registry for Canadians with ALS has been established in Calgary (21). This is a significant step forward for the Canadian ALS patients and research communities that will result in advancements in our understanding of ALS in Canada (21). While I prepared the thesis, a small scale (109 ALS patients since 1985) epidemiological surveillance study in Quebec reported a significant increase in the incidence rate of ALS during the 2005-2009 period compared with the previous periods. This increase is due to a significant increase in the incidence rate among the ≥65 years old group, from 7.38 per 100,000 persons/year (CI 95% 2.88-6.48) during 1985-2004 period to 12.22 (CI 95% 7.43-17.02) during 2005-2009 period (59).

2.3. Diagnosis: According to literature, ALS cases are classified into two categories: FALS (implied it is inherited) and SALS (implied it is associated with environmental risk factors). This clinic classification for ALS cases was still widely used for the convenience of clinical counselling, although it apparently is not accurate, and out-dated (41). Most ALS cases (95%) occur sporadically, and, therefore, belong to SALS category. However, about 10% of SALS cases are caused by monogenic mutations (3), partially due to low inheritance penetrance to all monogenic mutations and incomplete family history (60). Consensus of a clinical definition of FALS has not been reached as of yet. It has been suggested that FALS could be defined as in a family with two or more ALS affected members within first and second degree relatives, agreed with this definition by 38% of neurologist (61). There is also a question as to how to define a family, specifically with respect to the inclusion of first-degree relatives only or the inclusion of secondary-degree family members, even remote family members (60). A large retrospective study found that the RR of FALS was increased only among first and second degree relatives, not among third-degree relatives (62). I would suggest that familial clustering of FALS was limited within first and second degree relatives.

The diagnosis of ALS usually takes about one year from the onset of clinical signs. Because there is no specific test that can be used to diagnose ALS, diagnosis relies on differential diagnosis and the presence of unique clinical signs, specifically irreversible disease progression limited to within the skeletal muscles. In England ALS is diagnosed as motor neuron disease (MND, or motoneurone disease), a mainly clinical diagnosis.

Apparently ALS is a pathological diagnosis post-mortem. Spinal lateral sclerosis is a unique microscopic pathological change in ALS patients, which is formed by protein aggregation (cellular inclusion) in motor neuron cells. Clinical diagnostic criteria for ALS were proposed by the World Neurology Federation in 1994, and revised at least twice since then (in 2000 and 2008 respectively) (38). These criteria are referred to as EI Escorial criteria. According to these criteria, ALS cases could be diagnosed into three categories: definite, probable, or possible ALS. The WHO categorized ALS as a major subset of MND, but all subsets of MND share the same ICD code. Therefore, in literature, MND and ALS are often interchangeable. Since ALS is a rare neuron degenerative condition with unknown aetiology largely, there is no disease specific clinical diagnostic biomarker available for assisting the diagnosis. Consequently, misdiagnosis or misclassification is common, accounting for about 10% of total ALS cases (38).

2.4. Pathology and disease mechanisms: ALS can present either as a sporadic or familial form, but the clinical manifestations and prognosis are not markedly different, although the age of onset was slightly younger among FALS cases (63), which account for about 5% of total ALS cases (64), are defined as at least two ALS affected members were identified in a family's biological members (first degree relatives) (61). A large proportion of FALS is caused by monogenic mutations and is transmitted in a classic Mendelian fashion: these mutations occur in about two dozen genes that have been associated with ALS, and they collectively are responsible for about 50-80% of all FALS cases (64-68,68-72), but only for about 10% of SALS cases (3). The first dominant transmitted mutation was identified in the early 1990s in the *SOD1* gene, and has been replicated in a transgenic mouse model (73). More than 160 mutations in the *SOD1* gene have been reported in about 20% of FALS cases (74). However, ALS related *SOD1* mutations do not knock out its enzyme activity, but gain an adverse property, which is associated with the aggregation of intracellular proteins (75). The absence or overexpression of the *SOD1* gene in mice does not initiate typical ALS disease (73). However, the transgenic mouse with the human *SOD1* mutation develops ALS at 2 to 6 months of age, with typical ALS clinical symptoms and pathological changes (76-78). The mutations in *SOD1* gene display ethnic differences. For example, the AV4 mutation is most common in the United States (79,80), while the H46R mutation in *SOD1* is

most common in Japanese FALS patients. It has been noted that the H46R *SOD1* mutant protein has essentially no enzyme activity: the survival time for patients with this type of mutation is extremely long, often more than 12 years (81).

Given the fact that ALS affected motor neurons accumulate large amounts of ubiquitinated cellular proteins as a unique common pathological change in all ALS patients, it has been hypothesized that impaired cellular protein digestion is a common disease pathway in all ALS cases, along with impaired mitochondria functions (82,83). Recently, mutations of the *TDP-43* and *FUS*/TLS genes, both of which are DNA/RNA binding proteins, were found to be associated with a risk of FALS (84,85). *TDP-43* mutations are the second most common mutations observed in some FALS and SALS patients after *SOD1*, with up to 5% of total FALS cases and about 2% of SALS having *TDP-43* mutations. In ALS affected motor neurons, *TDP-43* positive (coexisting with *SOD1* protein) or *FUS*/TLS positive protein aggregate inclusions have been observed (85). A large scale case-control study showed that *FUS*/*TLS* was responsible for about 1% of all SALS cases (85-87). It has been speculated that both gene products are involved in protein digestion pathways, including endoplasmic reticulum (ER) stress (87), which is associated with breakdown of unfolded protein in the ER. Transgenic human *SOD1*, which still retains enzyme bioactivity, but has reduced binding affinity with the target, was shown to be associated with protein unfolding, thereby generating ER stress (ER stress) (87). Thus, the hypothesis is that both genes affect the rate of target protein turnover by regulating target gene RNA degradation or stability. The mutated *SOD1* RNA may be more stable, or the mutated *SOD1* gene could generate more abnormal unfolded protein, such that the affected host cells undergo constant ER stress (88), and eventually become shrunken (after undergoing apoptosis). However, the aetiology of ALS might not be so simple, as *SOD1* transgenic mice are more resistant to the development of ALS when gene *XBP1*, whose product plays an important role in mediating the response from unfolded proteins (or ER stress), is removed (89). Therefore, the formation of protein aggregates in ALS and how it affects the motor neurons, is still not understood.

2.5. Potential risk factors responsible for the occurrence of SALS: ALS is a multifactorial degenerative condition of motor neurons. Here we briefly summarized all related risk factors that have been investigated in the literature. Temporal (90,91),

geographical (90-92), and even occupational clustering (93) have been reported in almost every country in which ALS has been described; this type of clustering is commonly associated with a possible environmental risk factor.

2.5.1. Demographic risk factors

2.5.1.1. Age and sex: ALS incidence increases with age, peaking around 50-70 years of age (46,47). Interestingly, the incidence of ALS declines rapidly after this peak, suggesting that ALS is not only an age-related neurodegenerative condition. ALS affects males more frequently than females in various regions worldwide (55), whether or not this apparent gender difference is due to gender-specific biological factors is unknown.

2.5.1.2. Ethnicity: It is now widely accepted that the incidence of ALS is similar across white populations, including those in Europe, Northern America, and Israel (45). These observations indicated that race related risk factors might play a more important role than environmental factors in the development of SALS. The adjusted incidence among peoples with different ethnic backgrounds aged 45-74 years ever reported worldwide was within a range of 0.8-5.7 per 100, 000 persons per year, standardized against the US population in the year 2000 (45). The lowest standardized incidence rate was observed in Asian (45). Within the United States, several incidence and mortality studies have confirmed lower ALS frequency among African American and Hispanic populations than among non-Hispanic Whites (91,94). However, the range mentioned above might not be accurate, depending on the study, since even in same country, the standard rate difference could be as high as 2-fold (45). The race related risk factors for ALS responsible for this variation remain largely unknown.

The number of ALS cases believed to be caused by monogenic gene mutations represents a small proportion of total ALS cases. Ethnic difference has been identified in at least two ALS mutation genes. *C9orf72* mutation has mainly been identified in Whites, and with very low frequency among ALS patients in Japanese backgrounds (95-97). This repeat was not observed in mainland Chinese ALS patients (98), but with fairly high frequency among Taiwanese with Han ethnic origin (99). *SOD1* gene mutation profile in Whites is different from Japanese ALS patients (79-81).

2.5.2. Genetic risk factors for ALS onset: Three types of genetic risk factors related to ALS have been described. The first type is the monogenic mutations with Mendelian inherited traits. More than 20 mutated genes related to ALS have been identified, and this list keeps growing. The first identified most common mutated gene across all ethnicities is *SOD1*. The second identified most common mutated gene with Mendelian inherited traits is hexanucleotide expansions in the *C9orf72*. These types of *C9orf72* mutations are mainly identified in Whites with FALS, but they are rare in Chinese and Japanese (41). The second type of genetic factor is gene variants or gene polymorphisms. This type of gene changes alone would not cause ALS unless it interacts with other gene variants, or environmental factors. The third type of genetic factor includes epigenetic risk factors (100-102). The expression of the genes governing the normal functions of motor neurons could be modulated by environmental factors through DNA methylation (i.e., nucleotide cytosine) without causing gene sequence change. This kind of gene modulations by environmental risk factors may also be observed at the DNA, RNA, and protein levels.

2.5.2.1. Genetic factors with a Mendelian inheritance manner. Although ALS is a rare degeneration condition of motor neurons, more than 20 mutated genes linking to the occurrence of ALS in a Mendelian inheritance manner have been identified. This list is still growing. Unlike most other inherited diseases, the onset time for most inherited ALS cases usually happens at late life time, around 50 years old (calculated from ALS online website (10) and the age at onset could be as late as aged 85 years (103). The age at onset of inherited ALS might be subject to modification by environmental factors. Therefore, mostly for this type of genes, the inheritance penetration is much lower than 100%.

It should be advised that not all FALS cases are caused by this type of gene mutations, environmental risk factors also could cause familial aggregation of ALS cases (104-110). On the other hand, a small proportion of SALS cases are associated with this type of mutations (3). In addition, a few of mutated genes could cause the onset of ALS as early as less than 1 year old (i.e., ALS2; (10)). The newly occurred monogenic mutations might be arisen due to environmental mutagens.

2.5.2.2. Genetic factors that make carriers predisposed to ALS. Gene variants and gene polymorphisms have been a hot research topic in ALS genetic study area for a decade. More than 100 genes and their variants (more than 370 polymorphisms or SNPs) have been investigated in at least 130 studies over last decade (10). Twenty seven genes have been found to be associated with ALS in at least one study for at least one variant. Among the different studies exploring genetic factors, 11 gene variants have been studied in four articles (10), and summarized by meta-analysis. The abnormal expression of this type of genes alone is not sufficient to trigger the phenotype associated onset of ALS. It requires the presence of appropriate environmental risk factors or other genetic risk factors that make the individuals susceptible to ALS. Regardless of the directions of associations found via meta-analysis, none of the associations was uniformly consistent in all related studies. Three genes (*PON1*, *VEGF*, and *HEF*) have been widely investigated, but review of this literature suggests of a weak associations with ALS in large scale studies combined with meta-analyses published in peer-reviewed journals (111,112). The association identified in small scale studies was often not confirmed in studies of large scale (113,113-116). These conflicting results have emerged from research groups with similar background (117,118). Therefore, more studies with better study design in a large ALS cohort are required. I estimated that at least three thousand ALS cases were required for this type of studies (assuming the prevalence of the variant is 0.05, and the RR is 1.25, power is set at 0.8, with a significance level of 0.05); most of existing observational studies did not achieve this sample size.

2.5.2.3. Epigenetic factors: Epigenetics is a subject studying gene modification (i.e., methylation) with intact DNA sequence via environmental or genetic risk factors. About 90% of all ALS cases are associated with previous exposure to some environmental risk factors, and the penetrance of monogenic gene mutations associated with ALS is also subject to modulation by environmental factors, thus, epigenetic risk factors may play a more important role in the development and progression of ALS. The role of epigenetic factors in the development of ALS has only recently been proposed, demonstrated on the association between exposure to heavy metals and the risk of ALS (101). However, work on epigenetic risk factors remains in its early stages. During the course of preparing my thesis, two more studies about epigenetic risk factors for ALS were published. Global methylation and hydroxymethylation of the genes responsible for inflammation response were identified in

post-mortem SALS spinal cord but not in whole blood (102). Most compelling evidence showing that epigenetic trimethylation of lysine residues within histones H3 and H4, a novel mechanism involved in reducing *C9orf72* mRNA expression in expanded repeat carriers, was recently provided (119). With the advance of molecular biology the role of epigenetic risk factors in the development of ALS will be elucidated.

2.5.3. Environmental factors: Many environmental factors have been found to be associated with the occurrence of ALS, but no definitive causative link has ever been consistently established (68,100,101,120-122). The following risk factors have been repeatedly found to be associated with the development of ALS: agricultural chemicals/pesticides (123-125), head injury/trauma (126-129), heavy metals (lead, mercury, selenium) (101,120,130), cigarette smoking (19,131,132), electro-magnetic fields (133,134), and intense physical activities including participation in professional sports, blue collar related work (135,136). Scattered reports have also associated ALS with low levels of cholesterol, including using cholesterol lowering drugs, viral infections, and abnormal immune responses (137,138). Here I will discuss these factors in more details below.

2.5.3.1. Living in rural area, or previous exposure to pesticides including agricultural chemicals was associated with ALS: Two recent published meta-analyses independently summarised most existing articles related to the risk of ALS among individuals who had been previously exposed to pesticides (139,140). Both analyses essentially drew similar conclusion based on OR estimates (OR=1.90 and 1.88 respectively, exposed/non-exposed) because both meta-analyses were based on the same set of publications, except one additional cohort study was included in the second meta-analysis (140). We proposed and conducted similar analyses two years ago, in present thesis we updated and extended the meta-analyses by adding additional observational studies, and systematically reviewed scientific evidence from the aspects of genetics, toxicology, epidemiology, and clinics. More detailed discussion about this topic could be found in Chapter 5, manuscript 4.

2.5.3.2. Exposure to solvents: Nurses, laboratory technicians, machine operators/mechanics, cleaners, and workers in textile and shoes factories, and hairdressers, have been found to be at higher risk of ALS in many observational epidemiological studies (141-152). However, these studies have neither given a definition for what is solvent, nor used solvents as a primary predictive risk factor to study this association. Organic solvents are lipophilic substances, with the potential of disrupting the normal structure and function of neuron cells. Therefore it is biological plausible for considering exposure to solvents a risk factor for ALS. Our meta-analysis revealed that previous exposure to solvents was associated with ALS [random effects, OR=1.43(1.10-1.86)] (Appendix B, additional results).

2.5.3.4. Previous exposure to formaldehyde: Increased relative risk of ALS among adults who have ever been exposed to formaldehyde via their occupation [RR=1.34, 95%CI: 0.93-1.92) for full cohorts, or (RR=2.47, 95%CI: 1.58-3.86) for restricted cohort] has been reported (149). A strong dose–response relation between years of formaldehyde exposure and ALS mortality (referral, no exposure; OR=1.5, <4 years; OR=1.5, 4-10 years; OR=4.1, >10 years; trend p=0.0004) was identified, indicating that exposure to formaldehyde may be associated with the development of ALS.

2.5.3.5. Antecedent exposure to heavy metals was associated with an increased risk of developing ALS: Given the causative link between *SOD1* (zinc/copper regulated superoxide degradation enzyme) mutations and ALS, modulation of normal *SOD1* function by heavy metals is plausible. Indeed, exposure to heavy metals has been shown to increase the risk of developing ALS (12,120,121,153-160). The most widely studied heavy metals in ALS patients are lead and mercury, both of which have been shown to be neuron toxicants both in *in vitro* or *in vivo* studies, due to the accumulation of toxicity in neuron tissues (161). In addition, in an ALS animal model, exposure to zinc either hastened or delayed the time of onset of ALS symptoms (162,163); more studies are needed to clarify the role of neurotoxicity in the pathogenesis of ALS. There is also evidence of a positive relationship between environmental selenium and ALS incidence (164). The intake of magnesium was found not being associated with ALS in five cohort studies (165). Detailed summary regarding the association between exposure to heavy metals and ALS could be found in many generic reviews (154,155).

Mercury: The neurotoxic effects of mercury in humans and animals are well documented. *In vitro* experiments have shown that mercury damages the axons of neuronal cells, a typical pathological change of ALS neuronal degeneration. There have been anecdotal reports of ALS being associated with exposure to mercury (101,120,166). However, the relationship between the risk of ALS and exposure to mercury remains to be established in analytical epidemiological studies. Chronic exposure to methyl-mercury induced early onset of hind limb weakness in a transgenic ALS mouse model, indicating that methyl-mercury promotes the progress of ALS (101), and may even increase the risk of occurrence of ALS in humans. Previous exposure to mercury (unexposed/exposed: cases=69/11, controls=76/2 p=0.011) might be associated with ALS onset (167), and the mercury content in hair of ALS patients was higher than in controls, while the selenium content was lower (168). However, the higher mercury content in hair needs to be confirmed through further studies (169).

Lead: The association of previous exposure to lead with ALS has been studied extensively in case reports and observational epidemiological investigations. These observations are described in this thesis, after having searched, collated and reviewed the articles related to previous exposure to lead. The association of ALS with previous exposure to lead was synthesized with meta-analysis method based on the selected high quality observational studies, and the attributable risk of developing ALS following exposure to lead was also estimated in the thesis. More details could be found in Chapter 4, manuscript two.

2.5.4. Exposure to electromagnetic field may not be associated with ALS, but electronic shock may be: Early epidemiological studies showed that occupational exposure to electromagnetic fields was associated with a higher risk of ALS (133). However, animal experiments have failed to confirm this association (120). A large prospective study from Switzerland found that magnetic field distance from hydro power-line was not associated with an altered risk of developing ALS (170). The association of occupational exposure to electromagnetic fields with ALS might be confounded by the electric shock (134,171). Our analysis showed that previous electric shock was associated with ALS {[random effects, OR=3.27(1.87-5.73)], Appendix B, other results)}.

2.5.5. Previous trauma can be considered a risk factor for ALS: Previous trauma affecting neurons has been associated with the development of ALS; these findings have been consistently reported from the first reported ALS cases (172). However, the association of previous trauma with ALS has never been well established, but the hypothesis keeps reappearing over the last century. Two small scale pathological studies from Mckee et al supported the association between brain injuries and ALS (173,174). However, two recent studies (one population based cohort study, and the other population based case-control study) argued that the traumas were the consequence of the ALS disease (175,176), not its cause. They suggested that the association of ALS with previous trauma was driven by reverse causality. However, our multiple meta-analyses argue against this reverse causality assumption. Our analyses not only demonstrated that previous trauma was associated with ALS [random effects, OR=1.73(1.35-1.92)], but also demonstrated that old trauma (occurred at least 5 years before ALS diagnosis) was associated with ALS [OR=1.40, 95%CI: 1.06-1.86, random effects. More detail results and discussion about this topic can be found in the results section of manuscript three. The most challenging question for this association is that how one event causing brain injury could lead to progressive degeneration of motor neurons in both cerebral hemispheres. A recent study has made a step towards addressing this issue (177).

2.5.6. Participating in professional/organized sports, but not regular sports or normal active physical activity is a risk factor for ALS: Physical activity was recently found to be associated with occurrence of SALS in Japan (49), inconsistent with a more comprehensive study from Veldink's group (136). Other studies showed that physical activity played a very minor role in the pathogenesis of ALS (136,144,178,179). One cross-sectional study showed that professional soccer players, but not professional basketball or cyclists, have a higher risk of developing ALS, indicating that physical activity alone may not be a risk factor for ALS (180).

2.5.7. Strenuous occupations as risk factors for ALS

2.5.7.1. Repetitive strenuous work: Early epidemiological studies especially in Italy and Britain found that the workers involved in strenuous occupations such as professional sport, industries related to leather process, construction, mining, hairdressing, cleaning, soldering,

and many other occupations required repetitive and heavy laborious work had a higher risk of developing ALS (125,142,151). No biological evidence showed that the repetitive use of voluntary muscles could damage the related motor neurons. Excess mortality due to ALS was reported among Italy professional soccer players (180-183), and in American football players (184), but not in the studied cyclists and basketball players, further indicating that prolonged intense exercise itself might not be a risk factor for ALS. A few studies also found that lower education was associated with ALS (185-187). The pooled OR from these three studies for lower education (workers with lower education are often involved in strenuous occupations) versus higher education among ALS and control subjects was 2.04, 95%CI: 1.58-2.62 (random effects model, see chapter 6 of this thesis), further indicating that repetitive work might be associated with ALS.

2.5.7.2. White collar workers: Most studies showed that among people involved in occupation primary industry (blue collar workers) was associated with increased risk for ALS. However, some other white collar related information technology occupations were also found to be associated with increased risk for ALS (150,188,189).

2.5.7.3. Fire fighters: Firefighters were overrepresented among the deaths due to ALS, compared to deaths from other causes [OR=1.95(1.28–3.07)] (190). This increased risk was also observed among firefight supervisors and fire inspection staff [OR=1.54(0.43-5.57), and 2.70(0.59-12.49) respectively], the wider variations might be due to small case cohorts in both groups (6, 5 cases respectively). The risk among other comparable personnel in firefighting stations was not increased. These results suggest that the hazards related to firefighting related jobs, rather than the occupation itself (stress) was associated with an increased risk for ALS. The excess death among firefighters in Ontario has been noticed (191). However, an appropriately conducted epidemiological study in Canada was not available in the literature. Studies have found that hypoxia-inducible pathway in ALS patients were impaired. The expression of *VEGF* and *VEGFR2* in motor neurons of spine was down regulated in ALS patients compared to controls subjects (192). In addition, the *VEGF* level in cerebrospinal from hypoxaemic patients with ALS was higher than in CFS from normoxaemic patients with ALS (193), or in patients' monocytes (194). These results suggest that hypoxia might be associated with ALS.

2.5.7.4. Military service: The risk for ALS among military service personnel was statistically higher than in controls [RR=1.53(1.12-2.09)] (195). However, military service itself was not a strong risk factor [RR=1.34(0.87-2.06), compared to control] for ALS, the RR among military service men for ever participating in war compared to never participating in war was significantly increased [RR=1.36 (1.00-1.71)], and the risk was increased with increased numbers of wars participated (RR=1.57, 1.74, 1.97 for participating in one, two or three or more wars respectively). These results indicate that war related risk factors (such as explosives containing organic phosphorus compounds, stress, and war related injury) are associated with an increased risk of ALS.

2.5.8. Life style

2.5.8.1. Good fitness associated with, but not caused ALS: At least three publications observed an association between good fitness and the risk for ALS. The first of this kind of publications showed that an inverse association between BMI and the incidence of ALS in college varsity athletes (196). Two subsequent studies confirmed this type of associations by showing that the incidence of ALS was less common in patients with cardiovascular disease (CAD) than in non-CAD patients (197,198). The BMI was lower, and physical strength was higher in ALS patients prior to the ALS disease onset (197-199). Here we should not be confused by the fact that high BMI is a protective factor against ALS progression (200). This might be simply due to the extra energy supply for the ALS patients whose food intake functions have been impaired.

2.5.8.2. Inactivity (hours/day): One case-control study found that previous daily inactive time among cases was much longer than the time among controls (average 16.9 versus 13.9 hours) (186). This was an unexpected observation, because many studies have found that previous physical activities were associated with increased risk for ALS (201). We do not know whether or not this result had been mistakenly presented in this publication.

2.5.8.3. Smoking: Early studies produced controversial conclusions about the association between smoking and ALS (144,202-204). However, recent studies, mainly from Europe and United States, but not Japan, consistently showed that smoking was a risk factor for ALS (49,131,185,187,203-209). A statistically significant association between smoking and the

risk of ALS was reported only in females (RR=1.7; 95% CI 1.3 to 2.1), but not in males (RR= 0.9; 0.7 to 1.0) in a recent meta-analysis (19). It was estimated that the overall risk of ALS in smokers was about 1.3 fold (RR=1.28; 1.0-1.7) of non-smokers. This association of smoking with ALS supported the hypothesis that smoking might be a risk factor for ALS (132,210). More population based studies designed to elucidate the aetiology of ALS are required to verify these associations, since most studies included in this project were not designed specifically to investigate risk factors for ALS (131,185). A recent prospective population-based study concluded that smoking also affected the survival of female ALS, but not male ALS patients (206).

2.5.8.4. Alcohol drinking: A few studies have showed that light and moderate alcohol consumption was inversely associated with the incidence of dementia and Alzheimer (211). In contrast, a beneficial effect between alcohol drinking and ALS incidence had never been reported in previous studies. However, recently an observational study reported a negative association between alcohol drinking and ALS. It found that current alcohol consumption was associated with a reduced risk of ALS (incident patient group: odds ratio = 0.52, 95% CI: 0.40, 0.75) (212). Our meta-analysis revealed a negative association with fixed model only [OR= 0.86 (0.78-0.96)] with significant heterogeneity (I^2 = 67%, Appendix B for additional results).

2.5.8.5. Drinking coffee may play a protective role in the pathogenesis of ALS: A recent observational study found that, compared to none coffee drinkers, current coffee drinkers had reduced risk for ALS. The odds ratios for neurologic, non-neurologic, and GP controls were 0.7 (95% CI: 0.5, 1.0), 0.5 (95% CI: 0.3, 0.7), and 0.4 (95% CI: 0.2, 0.8), respectively (213), but the other two articles showed no significant association (202,214).

2.5.8.6. Vitamin E intake was inversely associated with the risk for ALS, but vitamin E supplement may not slow the ALS progression: High oxidative products have been suggested to be associated with ALS onset. In comparison with controls, oxidative products in plasma and SOD activity in erythrocytes of ALS patients were elevated (215,216). An increase in spinal cord lipid peroxidation in the FALS transgenic model, which precedes the onset of ultra-structural changes or clinical motor neuron disease, was also observed. The

greatest intensity of actual motor neuronal lipid peroxidative injury was associated with the active phase of ALS disease progression (217). Oxidant treatment causes a dose-dependent phenotype of apoptosis in cultured motor neurons (218). Higher intake of food rich in antioxidants such as fruit and vegetables may play a protective role against the development of ALS (219). These observations indicated that oxidation was associated with ALS. Therefore, intake of vitamin E, an antioxidant, might lead to a reduced risk for ALS.

Consistent with this hypothesis, a case control study showed that intake of vitamin E was associated with a reduced ALS risk (OR = 0.4, 95% CI = 0.2 to 0.7, p = 0.001) (220). A large pooled prospective study showed that an inverse dose-response between dietary vitamin E intake and ALS risk was evident in women, but not in men (221). A latest cohort study showed that compared to lower serum vitamin E individuals, the age-adjusted relative risk (RR) for ALS in subjects with higher Vitamin E was significantly lower 0.56 (95% confidence interval 0.32 - 0.99, p = 0.046) (222). These results suggest that intake of vitamin E may reduce the risk of developing ALS. However, supplementation of vitamin E in ALS patients might be inefficient for arresting the disease progression. After 12 months of treatment, vitamin E had no effect on the disease progression and survival time (223-225), indicating that the disease process could not be arrested by vitamin E alone.

2.5.8.7. Intake of fruit/vegetable: Two case-control studies from same Japanese authors reported an inverse association between intake of fruit/vegetable and the risk of ALS (49,219).

2.5.8.8. Chicken soup: A cohort study showed that the risk for ALS among the persons who consumed chicken soap versus the risk among the persons who have never consumed chicken soap was significantly lower after adjusted with age and smoking [RR=0.94 (0.66-1.34), 0.79 (0.55-1.13), and 0.58 (0.36-0.94) for one serving, 2-3 servings, and more than 4 servings per day respectively] with a statistical significant dose dependent reduction in risk with increasing consumption (P for trend is 0.0006) (214).

2.5.8.9. Glutamate: An early population based case-control study showed a positive association between consumption of glutamate and increased risk for ALS [RRad=3.2 (1.2-8.0)] (202).

2.5.9. Association with comorbidities

2.5.9.1. Infectious disease: High titers of enterovirus have been found in the spinal fluid of ALS patients in some (226-229), but not other studies (230,231). An association between polio virus infection and ALS has been also proposed (137). Theoretically, an immune response elicited by virus infection can cause paralysis of skeletal muscles in a tissue (motor neuron) specific manner. Because ALS specifically affects voluntary skeletal muscles, thus it is logical to speculate that virus infection may be a risk factor for ALS. Such speculation is partially supported by an observation of abnormal immune response in ALS patients (232). Although the hypothesis of viral infection and subsequent elicited immune response could be used to explain the specificity of ALS affected tissues, unfortunately, the link to virus infection for ALS has not been established with observational epidemiological studies (233).

2.5.9.2. Cardiovascular factors: Local vascular disruption is another hypothesis to interpret the specificity of the affected tissues in ALS patients. Vascular disruption in ALS patients has been observed (234,235). Variants of *VEGF*, a growth factor for vascular endothelial cells, have been associated with ALS (112,236-238). Beneficial vascular factors, such as nutrition factors vitamin E, cholesterol lowering drugs, and alcohol consumption also affect the progress of ALS (212,220,221,239). Lower LDL/HDL and lower BMI has been associated with an increased risk of ALS, and decreased survival in ALS patients in some (198,200,240-243), but not all studies (244). One cohort study found that the incidence of ALS among heart disease patients was lower than that in control population (240), further suggesting that the beneficial cardiovascular profile is negatively associated with ALS (198,201).

2.5.9.3. Impaired productivity in women: Impaired productivity had been observed among female ALS patients in one early study (93), and in two followed articles by the same first author (245,246). The impaired productivity is often associated with relatively higher testosterone but lower estrogen levels. We have known that the incidence of ALS in males

was higher than in females, therefore, the question about how sex hormones affect the onset of ALS needs to be explored. A recent case-control study from Holland found that an increase of the reproductive time-span by a year decreased the risk of ALS with an OR of 0.95 (p = 0.005) (247). Each year longer of the reproductive time-span [HR=0.90 (p = 0.01)] and lifetime endogenous estrogen exposure [HR=0.96 (p = 0.025)] were associated with a longer survival of ALS patients. These results indicate that longer exposure to female reproductive hormones has a neuron-protective effect on motor neurons.

2.5.9.4. Cancer: The overall risk for ALS among cancer survivors was not increased (RR=1.0), except the survivors of two rare cancer types. The standardized mortality ratio (SMR) was 1.6 (95%CI: 1.1-2.6) among melanoma survivors based 32 ALS cases; the SMR was 2.7 (95%CI: 1.1-5.7) among tongue tumour survivors, but based on only 7 ALS cases identified, indicating that no common causative pathway was shared between cancers and ALS (248). These observations were further confirmed based on larger datasets by same authors (249). ALS mortality was not significantly associated with the incidence of total cancers (SMR =1.00, 95%CI: 0.95-1.06). However, there is a similar significantly elevated risk of ALS death among survivors of melanoma (SMR =1.49, 95%CI: 1.17-1.85) and of tongue cancer (SMR = 2.57, 95%CI: 1.41-62) was further reported. In addition, a significantly reduced ALS death risk among prostate cancer survivors (SMR = 0.86, 95%CI: 0.76-0.96) was also noted. It is worth to note that the number of observed ALS cases among the survivors with smoking associated cancer was same as expected, further support the conclusion that smoking is not a strong risk factor for ALS (249).

2.5.9.5. Thyroid disease: Thyroid disease has also been observed to be associated with increased ALS risk (250). In fact, metabolism rate is higher among ALS patients (251).

2.5.9.6. Chronic neuron diseases: Different from previous thought**,** the cognitive ability was impaired in a large proportion of ALS patients (30). The ALS and FTD could be caused by same gene mutation (*C9orf72*), could coexist in same patient (33,252). Another coexist neuron disease pair was ALS/PDC, mainly observed in Guam and surrounding area (253). However, we do not know if these comorbidities could increase the risk of developing ALS.

2.5.9.7. Stress: Some stress such as the stress caused by the death of relatives may be associated with increased risk of developing ALS (49,254).

2.5.9.8. Type A: Type A human behaviour has been associated with increased risk for ALS (49).

2.5.10. Food toxins (toxicants): An extremely high incidence of ALS (about 50-100 fold above the average worldwide incidence) in indigenous Chamorro people of Guam was noted in 1950s and 1960s. A high incidence of ALS has also been reported in a few other pacific islands nearby, and in the Japanese KII peninsula (48,51,255). The high incidence of ALS was thought to be associated with the consumption of cycad seed and flying fox by the indigenous people (256-259). The clinical manifestations and neurological pathological changes of typical ALS-PDC with high doses of BMAA (the neurotoxicant methylaminoalanie) in monkeys were partially replicated (260-262). BMAA is a neurotoxic amino acid, which could be produced by most aquatic cyanobacteria in cycad seed. It exerts its neurotoxic effects via activation of glutamate metabotropic receptors (mGluR5 and mGluR1) and induction of oxidative stress (263). Cycad seed contaminated with the BMAA was used to make cycad flour, which was consumed by indigenous Guamanians. The toxicity of 'cycad seeds' was known to Guamanians, who removed the toxicants by multiple washing during preparation of the flour. Initially, this theory was not well received because animal experiments showed that the consumption of cycad seeds required inducing ALS in humans via cycad seed flour was much higher than the levels consumed by the Guamanians. However, this unproven hypothesis was revived 15 years later by the observation of BMAA bio-magnification in foxes, which were used to be consumed by the Chamorro peoples, by Cox et al (259), and the documentation of BMAA in brain tissues of ALS-PDC cases (264). The intake of BMAA has been used to explain ALS clusters in Gulf desert, and in New Hampshire (256,265,266). Over the last a few years, a few ecological studies have also showed the associations between the spatial distribution of BMAA and the incidence of ALS in France (267) and in USA (268). Clinical and pathological changes mimicking ALS in rats has been established following treatment with BMAA (269). Moreover, despite the return of the BMAA hypothesis, causal relationship remains to be established in a population based study.

The high incidence of ALS in the Pacific islands has declined over the last four decades, to the point where it is now close to the incidence in the rest of the world. This also has been associated with life style changes in Guam after America annexed Guam post World War II (270,271).

General summary: Although enormous efforts have been made by researchers, about 50% of FALS cases have not been associated with any genetic change in their genomes. Most researchers believe that more ALS genes will be identified. However, the remaining FALS cases may not be explained by monogenic genetic factors alone, since some ALS cases caused by environmental risk factors also can be present among same family members because they shared same environmental factors. Conjugal aggregation of ALS cases, which are apparently associated with life styles or environmental factors, has been frequently seen (106). In addition, it is expected that the recessive gene changes mostly do not lead to familial aggregation of ALS cases, but mainly present as a sporadic form. Therefore, it is not appropriate to claim that all FALS cases are caused by genetic factors. A few environmental risk factors (i.e., exposure to heavy metals, pesticides, solvents, and experience of trauma) have been repeatedly associated with SALS in many observational studies. Unfortunately, these risk factors have not been well verified. Our goal in this project is to identify the risk factors associated with SALS using systematic review and meta-analysis.

Chapter 3. Intermediate CAG repeat expansion in the *ATXN2* gene is a unique genetic risk factor for ALS–a systematic review and meta-analysis of observational studies

Ming-Dong Wang[1], James Gomes[1], Neil R. Cashman[2], Daniel Krewski[1] and Julian Little[1]

1. Department of Epidemiology and Community Medicine, Faculty of Medicine, University of Ottawa, Ontario, Canada, K1H 8M5

2. Department of Medicine, University of British Columbia, Vancouver, BC, Canada V5Z 1M9

Abstract

Amyotrophic lateral sclerosis (ALS) is a rare degenerative condition of the motor neurons. About 10% of ALS cases are linked to monogenic mutations, with the remainder thought to be due to environmental and other genetic risk factors (including variants of polymorphic genes). We examined the association between ALS and an intermediate CAG repeat expansion in the *ATXN2* gene using a meta-analytic approach. Observational studies were searched with relevant disease and gene terms from PubMed/MEDLINE, EMBASE, and PsycINFO from January 2010 through to January 2014. All identified articles were screened using disease terms, gene terms, population information, and CAG repeat information according to PRISMA guidelines. The final list of 17 articles was further

evaluated based on the study location, time period, and authors to exclude multiple use of the same study populations: 13 relevant articles were retained for this study. The meta-analysis revealed that the presence of an intermediate CAG repeat (30-33) in the *ATXN2* gene was associated with an increased risk of ALS [odds ratio (OR) =4.44, 95%CI: 2.91-6.76)] in white ALS patients. There was no significant difference in the association of this CAG intermediate repeat expansion in the *ATXN2* gene between familial ALS cases (OR=3.59, 1.58-8.17) and sporadic ALS cases (OR=3.16, 1.88-5.32). Published studies consistently showed that the intermediate CAG repeat length in *ATXN2* was associated with neither age at onset of ALS (P>0.05) nor survival time of ALS patients (P>0.05). These results indicate that the presence of intermediate CAG repeat expansion in the *ATXN2* gene is a specific genetic risk factor for ALS, unlike monogenic mutations with an autosomal dominant transmission mode, which cause a more severe phenotype of ALS, with a higher prevalence in familial ALS.

Key words: amyotrophic lateral sclerosis, ataxin-2, meta-analysis, systematic review, relative risk

Introduction

Amyotrophic lateral sclerosis (ALS) is a rare neurological degenerative condition of the motor neurons leading to paralysis of skeletal muscles, characterized by rapid irreversible progression in most cases (28). Although the causes of ALS are largely unknown, it is thought that genetic and environmental risk factors contribute roughly equally to the development of ALS (17,18). Over the last 20 years, about 10% of all ALS cases have been linked to monogenic mutations in one of 20 or so genes (74,272). Worldwide, the most common mutated gene related to ALS is *SOD1* (superoxide dismutase 1), which is responsible for about 2-5% of all ALS cases (273). Mutations in *SOD1* comprise the first genetic factor linked to ALS for which there is a valid animal model (274). The contribution of other types of genetic risk factors (polygenic gene mutations, variants of polymorphic genes) to the burden of ALS has also been investigated in over 130 studies involving about 400 variants in more than 100 genes (10,65,275). The abnormal expression of this type of gene variant alone is likely to be insufficient to initiate the onset of ALS; rather, exposure to

certain environmental agents is thought to render genetic variant carriers susceptible to ALS (9). To date, extensive studies have not revealed a definitive, replicable environmental risk factor for ALS, although many environmental risk factors, such as exposure to heavy metals, exposure to pesticides, exposure to solvent, intake of biological toxicants, history of head injury, smoking, and military service have been reported to be associated with ALS in some studies (12,13,67,100,120,155).

Variants of approximately 30 genes have been found to be associated with ALS development in at least one study, but none of these associations has been replicated in large scale analyses, even for the most credible gene variants for sporadic ALS, such as the Paraoxonase 1 polymorphism (111), the H63D polymorphism in *HFE* (114), and variants of the gene encoding vascular endothelial growth factor (112). Over the last few years, repeat expansions in two genes in ALS patients have been investigated intensively. One is the GGGGCC hexanucleotide repeat expansion in the upstream of the *C9orf72* coding region (276,277), which causes familial ALS (fALS) among whites and populations with a Spanish ethnic background (278), but is rare among fALS cases with other ethnic backgrounds (97,279,280). The evidence surrounding this particular relationship has been summarized by Turner et al (41). The second is the higher occurrence of intermediate CAG (coding for glutamine) repeats (polyglutamine, polyQ) in the 5 prime terminal of the *ATXN2* gene in ALS patients (281). However, there is currently no agreement about which range of the intermediate CAG repeat is associated with ALS, nor its relative prevalence in fALS and sALS cases. To address these issues, we examined the association between this intermediate CAG repeat expansion in the *ATXN2* gene and ALS using meta-analysis.

Method and materials

This systematic review and meta-analysis was conducted following the PRISMA guideline (25), major features of which are listed in supplemental document 1. Articles related to the association between ALS (disease terms: ALS, MND, amyotrophic lateral sclerosis, motor neuron disease, motoneurone disease, or Lou Gehrig's disease) and intermediate CAG repeat expansion in the *ATXN2* gene (gene terms: ataxin-2, *ATXN2*, *ATX2*, *SCA2, ASL13, TNRC13*, polyglutamine, polyQ, CAG repeat) were searched in MEDLINE,

EMBASE, PsycINFO, Pubmed, Hugenet and Google Scholar from January 1, 2010 through to January 20, 2014. The detailed search strategies are described in supplemental document 2. The retrieved articles were exported to Refworks for screening. First, duplicates were removed by comparing the authors and titles of adjacent records after sorting the articles by first author. Second, irrelevant articles were excluded by reading the titles and abstracts of records against our inclusion/exclusion criteria (at least one disease term and one gene term were required for inclusion). Third, relevant or ambiguous articles were retained for next level screening by examining the full PDF copy of the article. At this level of screening, letters to editors, commentaries, generic reviews, case reports, conference abstracts, mechanistic and animal studies were excluded by examining full PDF. Articles without one of the essential characteristics of the study population (study location, case recruitment time, and method of recruitment) were also excluded at this screening level. Fourth, the remaining articles were assessed by examining the authors, research groups, country of study, case ascertainment, population recruitment periods, analytic methods, results, and conclusions based on the previous publication (282). Since ALS is a rare neurological condition, care was taken to avoid inclusion of multiple articles with same study participants. When two or more papers were from the same group of authors, special attention was given to information on the centers involved in, and periods of recruitment of the study participants. Any article with duplicate uses of ALS patient populations or any ambiguous description about the studied population was excluded. Fifth, data from the final list of included articles were extracted (including first author, published year, country, CAG repeat information, case and control information, and main results) (Supplemental Table 1). The screening and data extraction steps were assessed by second reviewer, based on a random sample of 4 of 33 articles available at these steps. Finally, data were synthesized using meta-analytic approaches based on both random and fixed effects models (25). The synthetic odds ratio (OR), Tau^2, I^2 and χ^2 statistics were reported, and potential bias due to small study effects was examined using funnel plots. Comparisons among population subgroups defined by geographic region and types of ALS were also made.

Results

1. Studies selected for meta-analysis: A flow diagram summarizing article search, screening, selection, assessment, data extraction and analysis is given in Figure 1. No previous systematic review was identified, except one meta-analysis (283). Seventeen relevant articles were selected for inclusion in the present study (Supplemental Table 1). Three studies were excluded because of multiple uses of same populations. One study was designed to test the presence of intermediate CAG in ALS-FTLD and familial ALS only, and was also excluded from the meta-analysis. The data from remaining 13 articles (first author, published year, country, CAG repeat information, case and control information, main results), all of which followed a case-control design, were extracted and synthesized by meta-analysis.

2. Determination of the range of intermediate CAG repeats in the ATXN2 gene that is associated with ALS: Since Elden and colleagues reported that a 27-33 CAG repeat expansion in the *ATXN2* gene was associated with sporadic ALS in 2010 (281,284), about two dozen studies have sought to verify this association (283,285-297), investigate the mechanisms by which ALS may develop (298,299), or investigate different clinical manifestations in ALS patients carrying CAG repeat expansions in the *ATXN2* gene (298-302). The initial study focused on the range 27-33 in the number of CAG repeats, and determined this range as an intermediate CAG (PolyQ) repeat using ROC curves. However, no consensus has been reached regarding which CAG repeat range is associated with ALS (281,285,303,304).

To address this issue, we compared the CAG repeat range between ALS patients and controls based on the information from the 13 studies selected for inclusion in Figure 2 (283,285-297). A high frequency of 27Q repeats was noticed in ALS patients, Parkinson's disease (PD) patients, and controls in Europe and North America (Figure 2a), but not in Chinese ALS patients or healthy controls (Figure 2b), where the peak of 27 CAG repeats in the *ATXN*2 gene was essentially absent (295,296). These results indicated that the peak of 27 CAG repeats is not an ALS disease-specific CAG repeat, but might be a specific genomic marker of ethnicity (305). Although outside the scope of the present study, the higher rate of 24 or 25 CAG repeats in ALS patients (Figure 2a, 2b) may be relevant to the transmission of

new larger CAG repeats to the next generation. Based on the 9 studies in North America and Europe in which detailed repeat information could be identified (Supplemental Table 1), the pattern of CAG 30-33 repeats in the *ATXN2* gene in ALS patients appeared to be different than in controls (Figure 2a). These data suggest that 30-33 CAG repeats is associated with ALS among white populations. Ethnic variation appears to exist, with the major CAG repeat range associated with ALS appearing to be 31-36 in Chinese subjects (Figure 2b) (295,296). Meta-analyses were then conducted, with and without the two studies from China, to explore this association in quantitative terms.

3. The synthesized odds ratio (OR) estimate from multiple studies based on meta-analysis: The 13 studies included in the present meta-analysis involved a total of 154 positive carriers of 30-33 CAG repeats in the *ATXN2* gene among 9,042 ALS cases (including fALS cases with no known monogenic mutations), and 46 positive carriers of the same CAG repeats among 13,116 controls. The crude prevalence rate of the CAG repeats (1.70%) in ALS cases was significantly higher than in controls (0.35%, P<0.0001). Comparing cases to controls, the odds ratio (OR) for ALS in relation to the 30 – 33 CAG repeat sequence was estimated to be OR=3.93 (95% CI: 2.49-6.20) using a random effects model (Figure 3), with moderate heterogeneity across included studies as reflected by the I^2 statistic [35%, P=0.10]. Similar results (OR=3.60, 2.54-5.09) were obtained with a fixed effects model (data not shown).

When the two studies from China were excluded, heterogeneity decreased (I^2=13%). The remaining 11 articles reported a total of 134 positive carriers of 30-33 CAG repeats among 7,625 ALS cases (including sALS and fALS cases), and 37 positive carriers of the same CAG repeats among 12,555 controls (see Supplemental Table 1). Excluding these studies, the odds ratio was estimated to be 4.44 (2.91-6.76) using a random effects model (Figure 4). A similar estimate was obtained with a fixed effects model (data not shown). Funnel plots failed to identify evidence of small study effects (data not shown).

4. The prevalence of intermediate CAG repeat in the ATXN2 gene in fALS cases is not different from that in sALS cases: Based on the above analyses, we concluded that the intermediate 30-33 CAG repeat expansion in the *ATXN2* gene is a genetic risk factor for

ALS. Further analyses were undertaken in an attempt to compare differences in risk between fALS and sALS. High CAG repeat expansions (usually greater than 34) in coding regions of the *ATXN2* gene is the cause of spinocerebellar ataxia type 2 (SCAT2) (306), transmitted in an autosomal dominant manner. However, if intermediate CAG 30-33 repeat expansion of *ATXN2* also causes ALS in an autosomal dominant mode, then it might be expected that its prevalence in fALS cases would be higher than that in sALS cases (The frequency of *SOD1* mutations is higher in fALS than in sALS patients). To address this issue, we identified four included articles that provided relevant genomic information for fALS (defined as two or more ALS cases identified in a family) and sALS cases. Meta-analysis using a random effects model showed that the OR for the presence of the intermediate CAG repeat expansion in fALS cases [OR=3.59 (1.58- 8.17)] was not significantly higher than the OR in sALS cases [OR= 3.01 (1.77- 5.11)] (Figure 5). The pooled prevalence of the intermediate CAG repeats was 1.32% and 1.58% among sALS and fALS cases, respectively, which are not significantly different from each other (χ^2=0.25, p=0.62). There was no evidence of heterogeneity (I^2=0%) or publication bias across the four included studies. Similar results were also obtained with a fixed effects model (data not shown).

5. The CAG repeat length was associated with neither survival time nor the age at onset among ALS patients: Out of 13 included articles, 9 articles provided partial numerical data about the age of onset of ALS (116 ALS cases), disease duration (29 cases), and the corresponding CAG repeat numbers in *ATXN2* (27,31,35,36,38,40,43-45), linear regression analysis showed that the number of CAG repeats in the *ATXN2* gene was associated with neither the age at onset (R^2=0.004) nor the disease duration (R^2=0.02), a finding that differs from a previous analysis of a subset of the data considered here (281). No differences in disease progression between ALS patients with and without the intermediate CAG repeat expansion in *ATXN2* were noted.

Discussion and Conclusions

In this study, we synthesized data from published articles related to intermediate CAG repeat expansions in the *ATXN2* gene in individuals with ALS, and found that an intermediate CAG expansion with a range of 30-33 repeats was associated with an increased

risk of ALS. Since significant differences with respect to the prevalence of these expansions among fALS cases compared to sALS cases were not identified, heritability is unlike the dominant autosomal transmission mode of inheritance of SCAT2 observed for CAG expansions greater than 34 repeats in the *ATXN2* gene. The intermediate CAG repeat may have been inherited differently in relation to ALS (see discussion below). In addition, the presence of the intermediate CAG repeat expansions in *ATXN2* was not associated with either age at onset or survival time of ALS patients. Therefore, the intermediate CAG repeat expansion in the *ATXN2* gene may not be a simple modulator of ALS (304,307). We conclude that the intermediate CAG repeat expansion in *ATXN2* is a unique genetic risk factor for ALS.

High CAG repeat expansions (usually greater than 34 CAG repeat) in the *ATXN2* gene (306), transmitted in an autosomal dominant manner, is the cause of spinocerebellar ataxia type 2 (SCAT2), a progressive neurodegenerative disease of the cerebellum, brain stem and spinal cord, with a somewhat higher incidence in Cuba than elsewhere (283,297,308). The intermediate expansion of CAG repeats in SCAT2 cases has also been reported (309,310), and coincidence of ALS and SCAT2 patients in the same family has been observed (311), indicating a certain degree of overlap between SCAT2 and ALS with CAG repeat expansion in the *ATXN2* gene. The expansion of CAG repeat in *ATXN2* leads to the production of elongated polyglutamine (polyQ) in the corresponding protein. The locus of the *ATXN2* gene has been mapped to chromosome 12, but the function of the *ATXN2* genes product is not known (312). The CAG repeats in *ATXN2* gene are variable in size. The length of the allele in normal subjects was found to range from 14 to 31 repeats, with over 90% of normal subjects demonstrating alleles with 22 repeats (306). The SCAT2 disease allele usually increases its size when transmitted to successive generations, and the longer expansions associate with earlier onset and more severe SCAT2 disease in subsequent generations (313), different from the ALS cases with intermediate CAG repeat expansion (see section 3). Whether or not the intermediate repeat length of CAG (within the normal range, up to 31, and ≥33 for SCAT2) was associated with some disease conditions was unknown until Elden and colleagues (281) first linked the intermediate CAG repeat expansion (27-33) to ALS in 2010. Other follow-up reports generally confirmed this initial finding, but there is no

consensus with regards to which repeat length is associated with ALS. One study using ROC (receiver operating characteristic) curves showed that more than 29 CAG repeats in *ATXN2* was associated with an increased risk of ALS (291); however, other studies have linked ALS with greater than 30 CAG repeats in Italy (289), 30-35 CAG repeats in Germany (304), and ≥28 CAG repeats in Italy (285). Here, we used 30-33 repeats as the cut-off for association with ALS because the lack of differences between ALS cases and corresponding controls is absent when the CAG repeat in *ATXN2* is less than 29 or greater than 34. Nonetheless, this intermediate CAG range for ALS might vary in different studies due to ethnicity (295,296).

The products of the *ATXN2* gene with intermediate CAG repeat expansion may not act like a typical gene modulator (304). The first study conducted by Elden and colleagues suggested that intermediate CAG repeats were a modulator for ALS (281). Further experiments showed such repeats might regulate RNA processing (304) and intracellular vesicular trafficking (302). Importantly, *in vitro* experiments showed that the expression products of *ATXN2* with intermediate CAG repeats could interact with *FUS* (314), a DNA/RNA binding protein, which when mutated could cause fALS as a result of impaired RNA intracellular trafficking (314,315). In addition, *TDP-43* cytoplasmic inclusions in motor neurons of ALS patients harboring intermediate CAG repeats primarily showed skein-like or filamentous *TDP-43* pathology, whereas ALS cases without ataxin-2 polyQ expansions (n = 13) exhibited abundant large round *TDP-43* inclusions (300,301), and accumulated activated caspase 3 in motor neurons, an upstream event in the *TDP-43* disease pathway. The product of *ATXN2* with intermediate CAG repeat expansion could increase the presence of *TDP-43* in the cytoplasm in a RNA dependent manner (281). Moreover, plasma *TDP-43* was robustly associated with CAG repeat length (281). These biochemical observations indicated that the expression products of *ATXN2* with intermediate CAG expansions interact with two important genes (*TDP-43* and *FUS*) in ALS pathogenesis. However, it seems that ataxin-2 with intermediate polyQ does not function like a typical modulator of other genetic risk factors for ALS (specifically *TDP-43* and *FUS*), because the mutation of a modulator alone should not cause primary outcome of interest (ALS). Even if the expression products of *ATXN2* with intermediate CAG expansion were a modulator of *TDP-43* or *FUS*, it must be a weak or very specific modulator, because differences in clinical

features of ALS, such as age at onset or survival time, between the CAG repeat carriers and non-carriers have not been demonstrated (see section 3).

If the *ATXN2* gene with intermediate CAG repeat expansion associated with ALS were transmitted in an autosomal dominant manner, as in the case of *SOD1* mutations, or as in the case of SCAT2 for longer CAG repeat expansions, then we would expect that the prevalence of the intermediate CAG repeat expansion in the *ATXN2* gene be higher in fALS cases than in sALS cases, as is the case for other genetic risk factors for ALS, such as mutations in *SOD1* and *C9orf72*. Although studies from Cuba and the United States suggested a dominant mode of inheritance (281,308,311), our meta-analyses did not show a higher prevalence of the CAG intermediate repeat expansion in fALS cases, as compared to sALS cases. Therefore, the intermediate CAG repeat expansion in the *ATXN2* gene is not a typical independent classic genetic risk factor transmitted in an autosomal dominant manner.

Van Damme (292) described a consanguineous married couple, the father suffering from an ALS-like disease with two affected sons [elder brother (79 years old) with CAG repeat alleles 31:33, onset at 75 years, pedigree (middle son, 77 years old) with CAG repeat alleles 33:33, onset at 71 years] and a normal carrier younger brother (75 years) with 22:33 CAG repeat alleles. These observations suggest a recessive transmission mode, unlike a SCAT2 family with longer CAG repeats descried in the same article (292). The transmission of intermediate CAG repeat expansion of *ATXN2* gene might behave like the *Asp90Ala* mutation in *SOD1*, which was transmitted in a recessive manner in the North Scandinavian population, but in a dominant manner in other regions (316). The longer CAG repeat expansions for SCAT2 are transmitted in a dominant manner, whereas this is not the case for ALS with intermediate CAG repeat in *ATXN2*. The different modes of transmission might be dictated by the different nature of the CAG repeat expansions in ALS and SCAT2. Compared to the longer CAG repeats in SCAT2 patients, the unique feature in ALS patients with intermediate CAG repeats in the *ATXN2* gene is that the intermediate CAG repeats in ALS patients were interrupted by a CAA triplet in almost all ALS cases with this CAG intermediate repeat expansion (299). This observation has been confirmed by other similar observations (284). The CAA interruption would produce ataxin-2 protein with less toxicity, and the interruption stabilizes the CAG repeats (317). This type of triplet is less common in

longer CAG repeats in the *ATXN2* gene in SCAT2 patients (283,308,318,319). The CAA interruption may explain why the intermediate CAG repeat expansion in *ATXN2* is associated with ALS, and does not generally produce a mild type of SCAT2. Other mechanisms may also influence this comparison. For example, the co-occurrence of fALS with SCAT2, or with Parkinson disease, would lead to non-diagnosis of ALS in fALS (311,320,321), thus underestimating the occurrence of the intermediate CAG repeats among familial ALS cases.

Collectively, the results of the present study suggest that CAG intermediate (30-33) repeat expansions in the *ATXN2* gene is a genetic risk factor for ALS. During the course of this work, a meta-analysis of the association between intermediate CAG repeats and ALS for a family with 3 ALS cases linked to CAG repeat mutations in *ATXN2,* apparently with an autosomal dominant transmission mode, was reported from Cuba (283). This article adopted a similar inclusion and exclusion strategy, and identified many of the same studies as were used in the present meta-analysis, with the exception that we also included the two most recent published studies (285,295). However, that study compared the synthetic ORs to determine the cut-off for the intermediate CAG repeat expansion (≥30 CAG repeats) that associates with ALS, compared the range used in the present analysis (30-33 CAG repeats). Because they also included gene carriers with more than 34 CAG repeats (which usually causes SCAT2), the synthetic risk was artificially underestimated (OR=2.16), as compared to our estimate (OR=4.31). We argue that our strategy of determining the optimal range of the intermediate CAG repeats for ALS is preferable, because it is known that if the number of CAG repeats is greater than 34, then the repeat is more likely to be associated with SCAT2, rather than ALS (289). In our analyses, we also compared the age at onset and survival time in ALS patients with and without the intermediate CAG repeats, and found no differences either onset or survival.

Further studies are needed to verify our conclusion that intermediate CAG repeat expansion in the *ATXN2* gene is a unique genetic risk factor for ALS. Future research should focus on the mechanisms involved in the etiology of ALS among intermediate CAG repeat carriers, and explore the variation in repeats among ALS patients with different ethnic backgrounds.

Acknowledgement: The article search process was greatly helped by librarian Lindsey Sikora, and graduate student Mona Hersi.

Figures and legends

Figure 1.

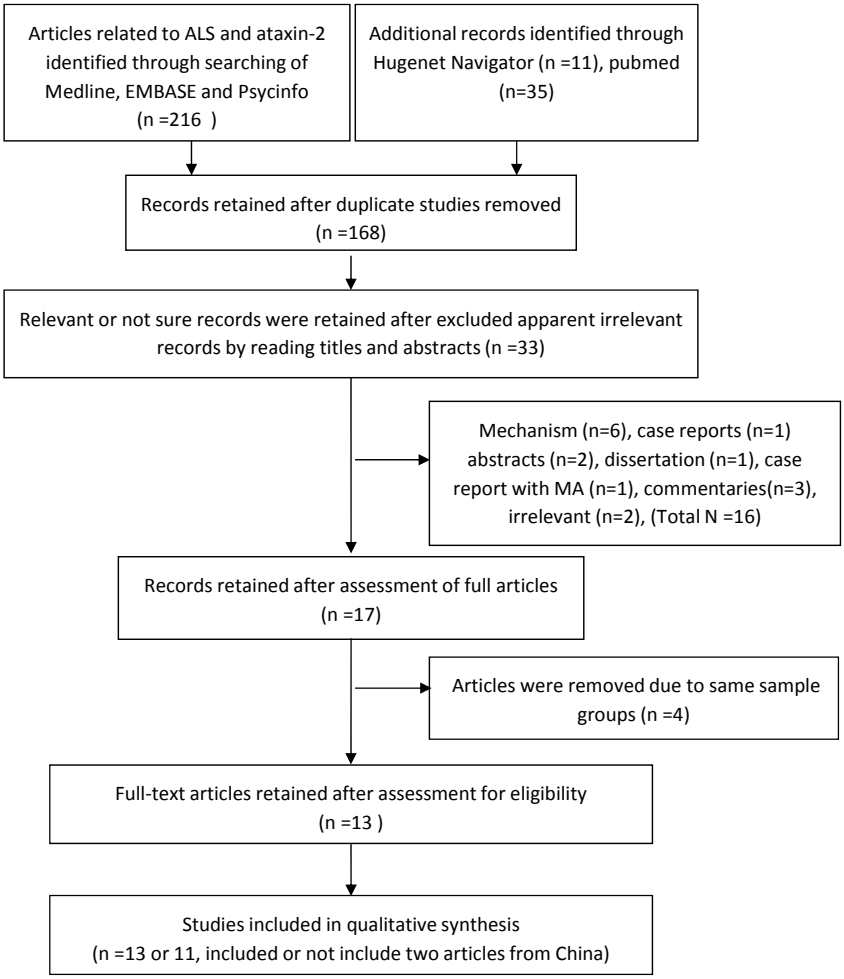

Figure 1. Flow chart for ataxin-2 and ALS related article search, screen, evaluation, and data analysis.

Figure 2.

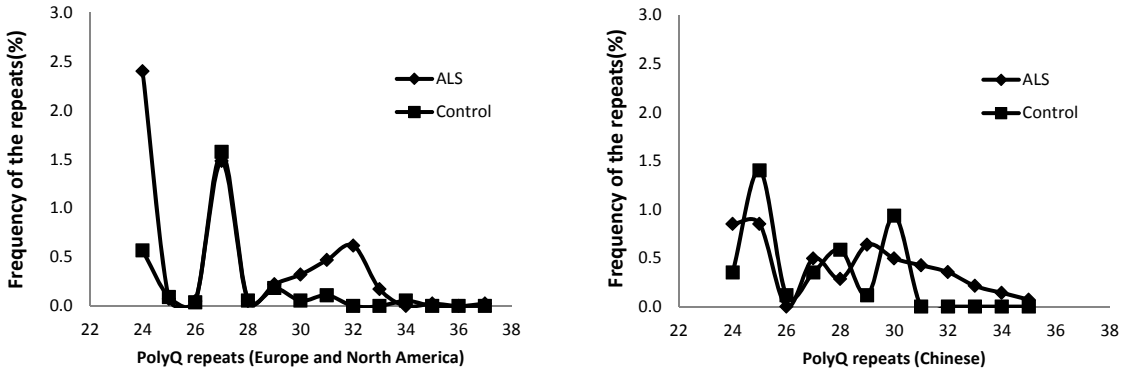

Figure 2. Identification of the differences of the presence of intermediate CAG repeats and potential ethnic variances in the ATXN2 gene among ALS and control subjects. The data about the number of CAG repeats in ALS and control subjects from 9 included studies in White and from 2 included studies from Chinese were summarized and plotted separately (left panel a; right panel b).

Figure 3.

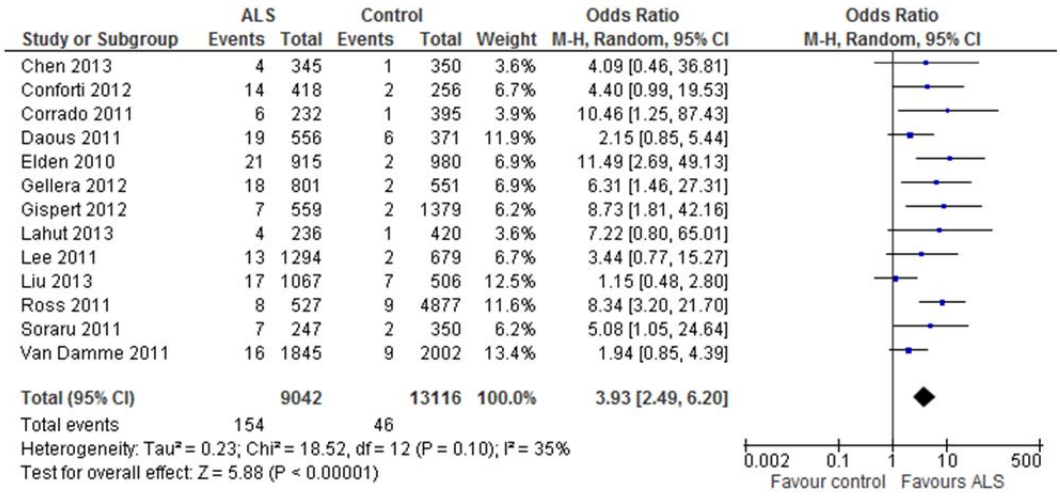

Figure 3. The presence of intermediate CAG 30-33 repeats in the ATXN2 gene is associated with ALS. The data of intermediate CAG 30-33 repeats in *ATXN2* were extracted from 13 included studies and the OR of intermediate CAG repeat among ALS and control subjects was synthesized with meta-analysis using random effects model. Similar results were also obtained when use fixed effects model (data not shown).

Figure 4.

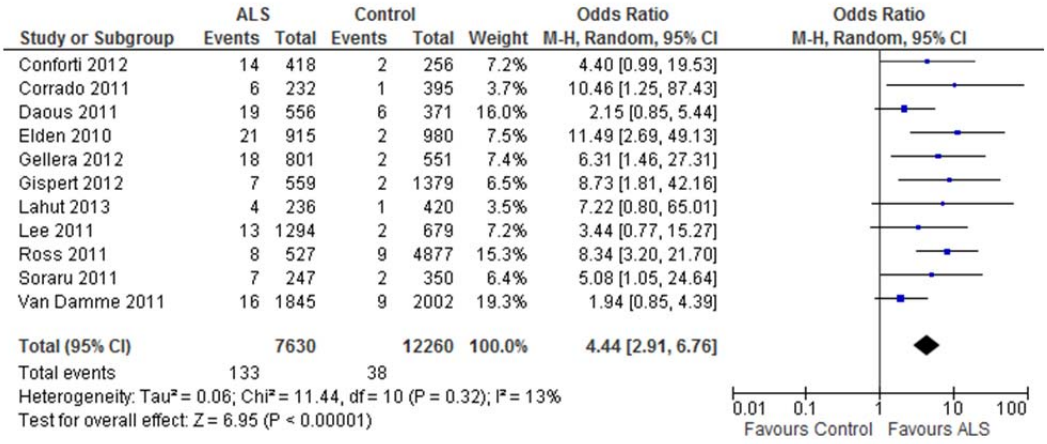

Figure 4. The heterogeneity among included studies was reduced dramatically after excluding two studies from China. This meta-analysis was conducted using same protocol as in Figure 3 except excluding two studies with patients with Chinese background. Please refer to Figure 3.

Figure 5.

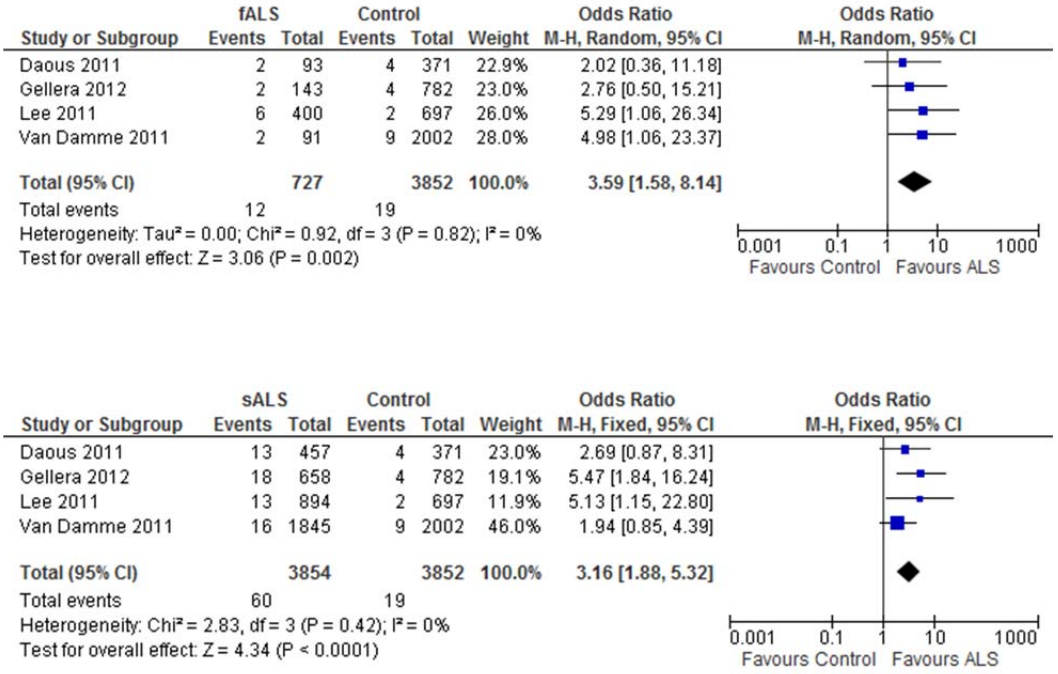

Figure 5. The synthesized OR of the presence of intermediate CAG repeats with meta-analysis is not different from FALS and SALS cases in the ATXN2 gene. The relative risks (OR) among familial ALS cases or sporadic ALS cases compared to controls were synthesized with meta-analysis using extracted data from 4 included case-control studies. The results from random effects model were presented. Similar results were also obtained when use fixed effects model (data not shown).

Search strategy

Search Criteria Used for the Identification of Articles on Ataxin-2 as a Risk Factor for ALS

The search strategy was employed to identify articles relevant to our investigation of the Ataxin-2 gene as a risk factor for amyotrophic lateral sclerosis (ALS) involved three separate searches of different bibliographic databases, each with unique search criteria designed specifically for those databases.

A. Medline, Psychinfo, and Embase

The search of these three databases involved the application of the following 21 search steps.

1. amyotrophic lateral sclerosis
2. motor neuron disease
3. motoneurone disease
4. Lou Gehrig's disease
5. als
6. mnd
7. 1 or 2 or 3 or 4 or 5 or 6
8. ataxin-2
9. *ATXN2*
10. atx2
11. sca2
12. asl13
13. tnrc13
14. polyglutamine
15. polyq
16. cag repeat
17. 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16
18. 7 and 17
19. limit 18 to english language
20. limit 19 to human
21. limit 20 to yr="2010 -Current"

A total of 216 potentially relevant articles were identified through this search.

B. Hugenet Navigator

1. First select ‘literature’ option, then enter disease term: amyotrophic lateral sclerosis
2. Filter with gene: *ATXN2*

A total of 11 potentially relevant articles were identified through this search.

C. Pubmed

(ALS, MND, amyotrophic lateral sclerosis, motor neuron disease, motorneurone disease, or Lou Gehrig's disease) and (ataxin-2, *ATXN2*, *ATX2*, *SCA2, ASL13, TNRC13*, polyglutamine, or polyQ).

A total of 45 articles were identified through this search. .

Supplemental Table 1: Summary of studies included in meta-analyses of the ATXN2 gene as a risk factor for ALS

Author, Year, Country	**ALS or MND Diagnosis**	**Sporadic, Familial sex, Case, Control and polyQ information**	**Main results and Conclusion**	Ref No.
Included studies				
Conforti, 2012, Italy	ALS, EI Escorial, exclude SOD1, TDP-43, ANG, FUS, C9ORF72 positive, Control matched with geographic	1. 405 sALS: 2. 13 fALS. 3. 296 control:	1. 57 out of 806 *ATXN1* alleles in sALS cohort harbored a >=32 polyQ repeat (7.07%), compared to 13 out of 544 NC alleles (2.38%, p=0.0001). OR=2.396(1.26-4.56) 2. For *ATXN2*, 22 X >=28Q in 808 sALS alleles (2.72%) and only 3 (0.5%) of 586 NC alleles (p=0.01). OR=5.832(1.71-9.78).	(285)
Corrado, 2011, Italy	ALS, EI Escorial. Screened SOD1, TDP-43, FUS, regionally matched controls	1. 232 ALS (219 sALS, 13 fALS): 1X24Q; 1X27Q, 0X28Q; 1X29Q; 0X30Q; 3X31Q; 1X32Q; 2X33Q; 1X37Q. 2. 395 controls: 3X24Q; 6X27Q, 1X28Q; 4X29Q; 1X30Q; 0X31Q; 0X32Q; 0X33Q; 0X37Q.	1. Significantly association. 2. No observation for symptoms of ataxia, dementia or other atypical features.	(284)
Daoud H, 2011, France/Quebec, Canada	1. ALS, EI Escorial, probable, or definite cases, diagnosed by ALS specialists. FALS were sod1, tardbp, fus, vapb, ang free. Neurological health controls matched by age, ethnicity. Not mentioned sex, and recruitment time.	1. 461 sALS: >=32, 9; 2. 95 fALS: >=32, 2; 3. 471 unrelated controls. Range from 19-32. 19-37 in patients. 24 of 471 healthy controls (5,1%) harboured 1 intermediate *ATXN2* allele(range 24-33), whereas 40 of 556 cases (7.2%) had one allele in that range (p=0.15). 4. Figure may help do scientific guess.	1, ROC showed that 27 cut-off gave best sensitivity/specificity. 19 from controls (4,1%), compared to 35 from cases(6.3%) within this range (>=27), no difference(p=0.09). The p becomes significant when compared within the range of >=29. 4 controls (0.8%), and 25 cases (18/461, 4.5%). OR=5.5(1.9-15.9). 7 out of 95 fALS (7.4%), 18 out of 461 sALS cases: OR for FALS was 9.29(2.66-32.4); for sALS is 4.74(1.59-14.13). When >=32 remove, the difference is still significant (14 from cases, 3 from controls, OR=4.03(1.15-14.11)) for the range between 29-31. 2. Length is not associated with age at onset (>=29) in cases were no >=29 cases.	(291)
Elden, 2010, USA	ALS, recruited in PA and NJ, no recruitment time, no case control design	1. 915 ALS. 6X24Q; 1X25Q; 0X26Q; 22X27Q; 1X28Q; 2X29Q; 4X30Q; 7X31Q; 8X32Q; 2X33Q. 2. 980 Controls: 4X24Q; 4X25Q; 2X26Q; 11X27Q; 0X28Q; 1X29Q; 0X30Q; 2X31Q; 0X32Q; 0X33Q	1. CAG intermediate repeat expansion is associated with ALS. 2. The repeat might be a modulator.	(281)
Gellera, 2012, Italy	ALS, EI Escorial, SOD1 free, for all, FALS also screened ANG, TDP-43, FUS, C9ORF72 (carriers of FALS cases-separated)	1. 658 SALS: 4 X 24-26Q; 10X27Q, 4X 29Q; 3X30Q; 7X31Q; 6X32Q; 2X33Q. No >33Q. 2. 41 FALS-G(carriers): 1 X 27-29Q; 1X30Q. No others 3. 102 FALS-Unknown: 2 X 24-26Q; 1X 27-29Q; 1X30Q. 4.231 Sporadic ataxic patients: 5X 24-26Q; 6X27-29Q; 2X30Q. No others. 4. 551 Health controls: 6X24-26Q; 18X27-29Q; 1X30Q; 1X31Q.	1. The frequency of ATXN2 alleles with 27-30 repeats was similar in SALS and control subjects. 2. Fifteen SALS subjects carried ≥ 31 CAG repeats. This difference was statistically significant (p = 0.0014). No alleles with ≥ 34 CAG were found. 3. In FALS, the distribution of ATXN2 alleles was similar to control subjects.	(289)

		No others.		
Gispert, 2012, Germany	ALS, European ALS clinics (diagnostic criteria),	1. 559 ALS (included 89 fALS, 1X 32Q): 1X24Q, 1X25Q; 0X26Q; 15X27Q; 0X28Q; 1X29Q; 3X30Q; 0X31Q; 3X32Q; 0X33Q; 0X34Q; 1X35Q. 2. 1378 controls: 9X24Q, 1X25Q; 0X26Q; 48X27Q; 1X28Q; 5X29Q; 1X30Q; 1X31Q; 0X32Q; 0X33Q; 1X34Q; 0X35Q. 3. 1142 PD: 6X24Q, 2X25Q; 1X26Q; 37X27Q; 1X28Q; 5X29Q; 0X30Q; 2X31Q; 1X32Q; 0X33Q; 0X34Q; 3X37Q; 1X39Q; 1X40Q.	1. In 559 sporadic ALS patients from Central Europe, the association of ATXN2 expansions (30≤polyQ≤35) with ALS was highly significant. 2. The study of 1490 patients with Parkinson's disease (PD) showed an enrichment of ATXN2 alleles 27/28 in a subgroup with familial cases, but the overall risk of sporadic PD was unchanged. 3. No association was found between polyQ expansions in Ataxin-3 (ATXN3) and ALS risk.	(304)
Lahut, 2012, Turkey	ALS, no other information	1. 236 ALS. 4X24Q; 1X25Q; 3X27Q; 1X28Q; 1X29Q; 1X31Q; 3X32Q. 2. 420 Healy controls. 3X24Q; 0X25Q; 1X27Q; 0X28Q; 0X29Q; 0X31Q; 0X32Q.	1. 15 ALS patients carrying SOD1, UBQLN2, OPTN, SPG11, or PLEKHG3 were intermediate repeat negative. 2. 4 X >31Q (1fALS, 3sALS). Could not find any information about total FALS cases. 2. Calculate >30 in this study. 3 out of 4 patients with 31 and 32Q had a single CAA interruption. Not in other ALS cases.	(298)
Lee T, 2011, Multiple European countries	ALS, EI Escorial, Controls matched by age and gender, who were either the spouses of ALS patients, healthy donors. Did not mentioned recruitment time	1. 400 fALS: >30, 6; 2. 894 sALS: >30. 7; 3.679 controls. 20 (2.9%) out of 679 controls harboured intermediate polyQ (range 27-30). 45 out of 1294 als patients (3.5%, range 27-35). For >30 repeat, no in 679 controls, but found 14 cases among 1294 ALS patients (p=0.0062).	1. No ataxia, dementia was observed in ALS patients. 2. No difference compared with and without repeat for age at onset, disease duration,. 3. Intermediate-length ataxin 2 polyQ repeat expansions are associated with increased risk for ALS also in the European cohort. The specific polyQ length cut-off, however, appears to vary between different populations, with longer repeat lengths showing a clear association.	(322)
Ross, USA, Canada, 2011	1. EI Escorial. Recruited from 2008-2010	1. 532 als: >=27Q, 33(6.2%); >=31Q, 8(1.5%). 2. 4877 control: >=27Q, 197(4.0%); >=31Q, 9(0.2%). 3. 642 FTD: >=27Q, 31(4.8%); >=31Q, 9(0.5%). 4. 1530 AD: >=27Q, 56(3.7%); >=31Q, 3(0.2%). 5. 514 PSP: >=27Q, 24(4.7%); >=31Q, 4(0.8%). 6.702 PD: >=27Q, 28(4.0%); >=31Q, 2(0.3%).	1. ALS, for >=27, OR=1.58(1.08-2.31); for >=31, OR=5.57(1.95-15.88). 2. FTD, for >=27, OR=1.20(0.82-1.76); for >=31Q, OR=1.94(0.51-7.37) 3. for AD, for >=27, OR=0.96(0.70-1.33); for >=31Q, OR=2.17(0.40-11.96) 4. For PSP, for >=27, OR=1.20(0.78-1.85); for >=31Q, OR=5.83(1.74-19.52) 5. For PD, for >=27, OR=0.95(0.63-1.43); for >=31Q, OR=0.93(0.19-4.51). Author speculated that long Q repeat in controls (9 controls) might be due to young age (reduced disease penetrance). SCA2 not CAA interruption?	(287)
Soraru, 2011, Italy	ALS, EI Escorial, recruited from 01 of 2004 to 08 of 2010. Did not mention control match	1. 247 ALS. >=24Q, 17(6.8%). 3 X24Q, 1X 26Q, 6X 27Q, 2X30Q, 1X31Q, 4X32Q 2. 256 controls: >=24Q, 6(2.3%). 1X24Q, 2X27Q, 1X28Q, 2X31Q.	1. Intermediate polyQ is more frequent in ALS patients, than in controls (p=0.026). 2. No difference was observed for age at onset, bulbar/spinal onset ratio, survival time, etc.	(307)
Van Damme, 2011,	1. ALS, EI Escorial. 1995-2010, neurological	1. 1845 SALS. >=32, 10(5 X 32, 2X33, 1X34, 1X36, 1X39; 0.5%); (31,4; 30, 5; 29,9; 28, 1; Scientific guess from fig. we can further guess 27 repeat)	1. p=0.0006 for repeat >=32 between ALS and control. No difference for <=31 (22-31, or 27-31 or 29-31). 2. ROC curve show a cutoff >=29 yield greatest sn, spn.28 out of	(292)

Belgium/Netherlands	conditions free normal controls	2. 103 fALS cases from 91 families. but sod1, fus, TARDBP, ANG free. 2/91 (2.2%) long repeat, 1/91, 31 repeat; 1/91, 33 repeat; 3. 2002 controls. Range, 16-31; 22, 90.1%; 23, 6.1%; 27, 1.7%; 31, 0.1% (heterozygous, 0.2%). (31,5; 30, 4; 29,7; 28, 1; Scientific guess from fig.)	ALS patients (1.5%) versus 16 out of 2002 controls (p=0.036, OR=1.92(1.04-3.64)). Combined with an American study (915 ALS, 980 controls), OR=2.93(1.73-4.98). 3. No association with survival, age at onset, site of onset. 4. Pedigree (33:33), onset at 71, and his 2 y elder als brother (31:33, onset at 75), normal brother (22:33) were described from consanguineous family. One of their parents was possible affected by ALS. No ataxia or cerebellar degeneration was found.	
Studies from China				
Chen, 2011, China	1. ALS, EI Escorial, 05-2004-06-2010. Excluded fALS, Community controls matched by age, sex, race from same period.	1. 345 sALS (254 spinal, 91 bulbar). >=24, 15; <24, 330; >=27, 12; <27, 333; >=28, 11; >=29, 8; >=31, 4. 2. 350 controls (17-30). >=24, 8; <24, 342; >=27, 4; <27, 346; >=28, 3; >=29, 2; >=31, 0. 3. Provide a table for comparing repeat length and clinic features	1. Mean age of onset, gender, and onset site between with and without *ATXN2*, no difference. 2. p=0.040 for >=27. 3. Mean age of onset (for >=31), is longer than (<31): 44.5±8.5y verus51.57±12.51, p-0.197	(296)
Liu, 2013, China	ALS, EI Escorial, whole China, did not state the recruitment time, no control match information	1. 1067 als: 4X26Q; 4X 27Q; 3X28Q; 6X29Q; 3X30Q; 17 X >30Q (6X31Q; 5X32Q; 3X33Q; 2X34Q; 1X35Q). 2. 506 healthy: 1X26Q; 2X 27Q; 4X28Q; 0X29Q; 7X30Q; 0X >30Q. 3> 6 fALS, no.	1. Association of ALS with ataxin-2 intermediate CAG repeats was confirmed. 2. No clinical manifestation associated with repeat observed.	(295)
Excluded article				
Van Langenhove, 2012, Belgium	No diagnostic, recruitment and control information.	1. 72 ALS cases, including 18 fALS. 27-33Q, 7; 30-33Q, 3; >31Q, 1 (33Q). No information for fALS alone. 2. 22 FTLD-ALS: 27-33Q, 1; >31Q, 0. 3. 270 FTLD. 27-33Q, 8; 4. 810 controls.27-33Q, 25; >31, 0.	1. Significant phenotype overlap between ALS and SCA2 was observed. 2. Intermediate repeat of polyQ is associated with fALS. 3. No similar association was identified for FTLS and FTLD-ALS.	(288)
Multiple use of same sample		Title	Main results	
Bonini, 2011, USA	Same as Elden	Model organisms reveal insight into human neurodegenerative disease: ataxin-2 intermediate-length polyglutamine expansions are a risk factor for ALS	1. For 27-33Q; 1.4% in control; 4.7% in ALS (including sALS and fALS). Significantly associated intermediate polyQ repeats with ALS. 2. Mentioned that the cut-off appeared dependent on the specific population. 3. This study used similar approaches as in Elden's paper	(286)
Lee T, 2011, USA	Same as Elden	Evaluating the prevalence of polyglutamine repeat expansions in amyotrophic lateral sclerosis	1. Assessed the polyQ lengths of ataxin 1, ataxin 3, ataxin 6, ataxin 7, TBP, atrophin 1,and huntingtin in several hundred patients with sporadic ALS and healthy controls. 2. Other than ataxin 2, we did not identify a significant association with the other polyQ genes and ALS	(290)

Yu Z, 2011, USA	Same as Elden	PolyQ repeat expansions in ATXN2 associated with ALS are CAA interrupted repeats	Expanded repeat alleles of 40 ALS patients and 9 long-repeat length controls were all interrupted, bearing 1–3 CAA codons within the CAG repeat.	(299)

Chapter 4. A meta-analysis of observational studies of the association between chronic exposure to lead and amyotrophic lateral sclerosis

Ming-Dong Wang, PhD, James Gomes, PhD, Neil R. Cashman, MD, Julian Little, PhD, and Daniel Krewski, PhD,

Author information

From the Department of Community Medicine and Epidemiology (Drs. Wang, Krewski, Little Gomes), Faculty of Medicine, University of Ottawa, Ontario, Canada, K1H 8M5; and Department of Medicine (Dr. Cashman), University of British Columbia, Vancouver, BC, Canada V5Z 1M9

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Authors Wang, Krewski, Cahsman, Little and Gomes have no relationships, conditions, or circumstances that present potential conflict of interest.

Running title: Meta-analysis of ALS and exposure to lead

Abstract:

Objective: The association between exposure to lead and the risk of developing amyotrophic lateral sclerosis (ALS) was examined through systematic review and meta-analyses of relevant epidemiological studies according to PRISMA guidelines.

Methods: Relevant studies were searched in multiple bibliographic databases through to September 2013; additional articles were tracked through PubMed until submission. All records were screened in DistillerSR, and included articles were evaluated with the modified Moose system.

Results: The risk of developing ALS among individuals with a history of exposure to lead was increased by 86% (OR=1.86, 95% CI 1.39-2.48) compared to controls, with no apparent heterogeneity across included studies [I^2 = 23%]. The maximum attributable risk of ALS due to exposure to lead was estimated to be 5%.

Conclusion: Previous exposure to lead may be a risk factor for ALS.

Introduction

Amyotrophic lateral sclerosis (ALS), a neurological disease term often used interchangeably with motor neuron disease (MND) in the literature, is a rare multifactorial degenerative condition of motor neurons characterized by rapid and irreversible progression. It presents either as a familial (fALS) or sporadic (sALS) form, accounting for 5% and 95% of all ALS cases, respectively (1). ALS begins with either a bulbar or spinal onset in one third and two thirds of ALS cases, respectively. The disease usually occurs in adults, with an average age at onset of 60 years (2), with the average age at onset being higher among bulbar as compared to spinal cases (2). Survival following diagnosis is about 2-3 years for bulbar onset cases and 3-5 years for spinal onset cases (2).

The causes of ALS remain largely unknown, except for a small proportion (about 10%) of cases (including both sALS and fALS cases) that are related to monogenic mutations (3). This type of monogenic mutation has been found in about two dozen genes (10,14,15,74). In white ALS patients, the most common monogenic mutated genes are *SOD1* and *C9orf72*; these mutations are responsible for 25% and 35%, respectively, of fALS cases and 2% and 6% of sALS cases (2-6). The mutation in *C9orf72,* which is rare in non-white populations (6), arose in Scandinavia several thousand years ago (7,8). However, most (90%) of sALS cases are believed to be caused by polygenic variants/polymorphisms, environmental risk factors, and perhaps stochastic factors that exert their influence only in genetically susceptible individuals (323). Environmental and genetic factors are thought to play equally important roles in the development of ALS (17,18). Although many polymorphic gene variants (such as *PON1* and *VEGF*) and environmental factors (such as pesticides, heavy metals, trauma, smoking, and electric shock) have been reported to be associated with of ALS, none have been conclusively determined to cause ALS (12,13,67,100,120,155).

Prior exposure to heavy metals, including lead, has long been suspected to be associated with an increased risk of ALS. Anecdotal case reports have associated the onset of ALS with exposure of several heavy metals, including selenium, mercury, lead, aluminum and magnesium (155). Epidemiological studies exploring the association between prior exposure to lead and ALS began about five decades ago, after a series of ALS cases with antecedent exposure to lead were

reported as early as 100 years ago (324-326). Since then, nearly two dozen observational epidemiological studies of ALS cases with retrospective exposure to lead have been conducted (130,141-144,146,147,150,152,167,186,326-335). Most studies clearly showed that occupational exposure to lead was associated with a higher risk of developing ALS (144,332,336), although some studies failed to observe a statistically significant association (144,167,331). The major challenge in linking lead and ALS is the retrospective ascertainment of historical lead exposures. Because lead levels in blood or other body fluids may not represent previous exposure to lead, it is perhaps not surprising that some previous studies did not observe differences in lead exposure between cases and controls (332).

A recent study of the spatial distribution of ALS showed a gradient in the incidence of ALS in the vicinity of a lead melting factory in a county of Missouri State (337). In a case-control study in Boston, Kamel and colleagues established a dose-response relationship between ALS and diverse measures of exposure to lead (143,186,332). A case-control study reported evidence of association consistent with a linear dose response relationship between blood lead levels and ALS (130). The purpose of the present study was to further evaluate the association between prior exposure to lead and diagnosis of ALS using systematic review and meta-analytic approaches.

Methods and materials

Search strategy

The search strategy was initially developed in Medline using search terms selected to identify relevant scientific publications, including systematic reviews, meta-analyses, and observational (case-control, cohort, and cross-sectional) studies (Supplemental document 1). Once the search strategy was sufficiently well developed, it was used to search other bibliographic databases, with minor modifications to adapt to the requirements of those databases. The search included disease relevant terms (amyotrophic lateral sclerosis, ALS, motor neuron disease, MND, or Lou Gehrig's disease), and terms of environmental risk factors in order to identified all environmental risk related studies (see supplemental document 1). The articles related to exposure to lead were further identified with lead exposure related terms (see below).

Databases searched

The following databases were searched through to September, 2013: Medline, PubMed, EMBASE, Toxline/toxnet, Ageline, Proquest (including dissertations), Psycinfo, and Google Scholar. Relevant articles were also hand-searched from reference lists and other databases, or through contacting to article authors. Similar articles were tracked with PubMed until the submission of this manuscript.

Screen and selection of retrieved articles

Duplicate articles are removed using Reference Manager by comparing authors and titles in adjacent references, after sorting references by author name. The retained articles were then screened in DistillerSR using predesigned screen forms. Level one screening was conducted by reading the titles in relation to the inclusion/exclusion criteria [English language, human study, relevant disease terms, relevant risk factors terms, and observational epidemiological study information (cohort, case-control, cross-sectional, but not an intervention study or a review or commentary)]. Level two screening was conducted by examining abstracts with respect to the inclusion exclusion criteria. Level three screening was conducted by examining the complete article and applying additional inclusion/exclusion criteria relating to population information, case ascertainment, environmental risk factors (military service, and studies with ALS from Guam only were excluded since the former one used selected population, and later one used the ALS samples associated with specific local risk factors), data analysis methods, and results had to have been presented] using two reviewers. During this step, if we found that an article had only fALS cases, or an article was with only an ecological study design, then the article was excluded. Following this step, the retained articles were examined to carefully identify terms related to exposure to lead (lead, heavy metal, solder, soldering, Pb, weld, or welding) by using 'find' function in the PDF file or reading through the methods and results sections of the article.

Quality assessment, data extraction and meta-analysis

Since ALS is a rare neurological condition, it is important to determine if information on the same sample of ALS patients has been reported in more than one article. If so, then the earliest article was retained for meta-analysis. In the present analysis, comparisons were based on

subjects ever-exposed versus never-exposed to lead through the occupational environment. Relevant information was then abstracted from the selected articles, including information about the population under study (at least 5 or more cases in each cell of a 2 x 2 contingency table), study period, country, response rate, population information for controls, diagnostic criteria, data analysis methods, risk factor information, and study results. A 5% of random sample of the studies from which data had been abstracted was verified by a second reviewer.

All articles selected for meta-analysis were evaluated using criteria previously used by our research team (23,24). Meta-analysis was used to compare the risk of ALS in occupationally exposed and non-exposed groups based on the results of the included studies. The odds ratio for ALS in relation to lead exposure was used as the summary measure of risk in these analyses. The primary analysis was based on random effects modelling, with a fixed effects model run as a secondary analysis using Reviewer Manager 5. Heterogeneity across included studies was estimated by Tau^2, Chi^2, and I^2. Forest plots and relevant supporting statistics were examined. Funnel plots (Begg's test) were used to evaluate possible publication bias. Meta-analysis for subgroups (based on disease subtype, sex, exposure to lead versus to heavy metals, adjusted risk estimate or the article quality) were considered if at least three articles are available for each category.

Maximum attributable risk of ALS due to previous exposure to lead

The combined prevalence data for meta-analysis was used to estimate the maximum attributable risk (MAR) due to previous exposure to lead among ALS cases (338). The assumptions underlying this calculation are: 1) the disease prevalence in the general population is low (usually <5%); 2) previous exposure to lead is causally associated with ALS; 3) ALS cases and controls included in the selected observational studies are representative of the total population of ALS patients and general population; and 4) all excess exposure events among ALS cases as compared to controls are responsible for the development of ALS among those who reported being exposed to lead. Under these assumptions, this attributable risk can be considered as the maximum attributable risk (MAR). The MAR is given by

$$MAR\ (\%) = 100 \times p*(r-1)/[p*r + (1-p)],$$

where p denotes the prevalence of exposure to lead among control subjects; r denotes the relative risk for ALS due to exposure to lead, estimated here by the odds ratio, OR, which is a close approximation to r when the OR is not large.

Results

Summary of literature search

The results of this systematic review and meta-analysis were reported according to the PRISMA guidelines (25). Details of the literature search, article screening, article evaluation, data extraction and data analyses as described in the methods section are summarized here in Figure 1. All of the selected articles are case-control studies, except for two large cohort studies (Supplemental table 1) (150,335). Of the 20 selected articles, four articles used the same ALS subjects; the earliest report was selected for inclusion in the meta-analysis (130,143,186,332), leaving articles for further evaluation. Of the 17 retained articles, eight articles used the risk term 'lead', four articles used the terms relating to exposure to 'heavy metals', and five articles did not provide primary prevalence data, but only summary risk estimates. The meta-analysis was therefore conducted with the eight articles specifically addressing the relative risk post occupational exposure to lead. Sensitivity analyses were conducted to evaluate the effect of including the four articles focussing on occupational exposure to heavy metals of any type, including lead, and the effect of the study quality.

Meta-analysis of association between previous exposure to lead and ALS

The results of meta-analyses revealed that the odds of developing ALS were significantly higher among subjects with a history of occupational exposure to lead than among unexposed subjects (OR=1.86, 95% CI: 1.39-2.48, based on a random effects model (Figure 2); the corresponding estimate based on a fixed effects model was similar, with no significant heterogeneity across included studies [$P = 0.25$, $I^2 = 23\%$], and with no apparent publication bias indicated by the funnel plots (data not shown). A increase in the relative risk of ALS was found based on meta-analysis of four articles using heavy metals as the risk factor, rather than lead (Figure 2). Combining the eight articles focusing on lead exposure with the four articles focusing

on exposure to heavy metals yields an OR of 2.13 (1.33-3.42). Similar results were obtained using fixed effects model (data not shown)

The quality of articles seems not significantly affect the conclusion

As indicated in Figure 2, the relative risk of ALS due to exposure to lead is not materially different from the relative risk due to exposure to heavy metals. We therefore used all 12 studies to evaluate if publication quality affects the estimate of risk. Based on the quality assessment of articles included in the meta-analysis, the articles were divided into two groups, representing articles with higher and lower quality, respectively. If two articles have the same quality score, then the newer article (publication date) was allocated to higher quality score group. The meta-analysis revealed that the publication quality did not significantly change the estimate of relative risk, although the relative risk derived from articles with higher quality [OR=1.75(1.30-2.36)] is slightly lower than the relatively risk derived from articles with lower quality [OR=2.25(1.57-3.24)].

The estimated risk calculated from adjusted estimates is somewhat attenuated

Of the 20 included studies, five articles provided adjusted risk estimates (adjusted for sex and age, for example) only (either RRs or ORs) either for the risk factor 'lead' or for the risk factor 'heavy metals' (144,147,150,152,335). Two other articles also provided adjusted risk estimates, along with the prevalence of exposure to lead among cases and controls. One of these two studies has been included in Figure 2 (333); the other used the same sample of patients as the article authored with Kamel (2002) (130), and was therefore excluded from the analysis. We also conducted an additional meta-analysis with the estimates from these seven studies to assess the degree of agreement with the results for the 20 studies in Figure 2. The results of this analysis indicate that the relative risk increased by about 40% [OR=1.41(1.21-1.65)] (Figure 4), slightly lower than the estimate from Figure 2 and with no heterogeneity (I^2=0) among the included studies or evidence of publication bias (Funnel plot, figure not shown).

Sex effect on the risk for ALS

Two studies provided risk information for males and females separately (141,146). Interestingly, the relative risk of ALS was higher in females than in males after exposure to lead,

based on self-reported information in both studies. However, in the one study that used an expert panel for exposure assessment, the relative risk in females was actually lower than in males (339).

Maximum attributable risk due to previous exposure to lead

A total of 180 ALS cases from a population of 1,152 individuals with ALS had previous exposure to lead, whereas a total 154 controls from 1,478 normal subjects had previous exposure to lead, based on the eight studies that formed the basis for the present meta-analysis. The MAR due to previous exposure to lead is calculated based on these four values using the formula given in the methods section. The value of p is 0.1042 = 154/1,478, and the value of r is 1.4996 =[(180/1152)/(154/1478)]. The maximum attributable risk (MAR) is then 5.0% [0.1042*0.4996/(0.1042*1.4996+0.8958)]x100% of total ALS cases. This suggests that up to 58 of the total of 1,152 ALS cases from all included studies might have been caused by previous exposure to lead.

Discussion

The present meta-analysis of eight case-control studies indicates that the risk of ALS is increased by 86% following occupational exposure to lead, compared to unexposed controls. The estimated risk for ALS due to exposure to lead is not materially different from that calculated based on exposure to heavy metals. In addition, we found that the risk estimated based on the articles with low quality is not significantly different from the risk estimated based on the articles with high quality, although the risk is estimated to be slightly higher using articles of lower quality, as compared to those of higher quality (Figure 3). The available data suggest that up to 5% of all sporadic ALS cases may be attributable to occupational exposure to lead, although the actual attributable fraction could be somewhat lower because of the assumptions involved in this calculation. Nonetheless, our results suggest that previous exposure to lead in the occupational environment is a significant risk factor for ALS.

Lead is toxic to motor neurons in humans

Motor neuron toxicity in humans following exposure to lead was recognized more than a century ago (326). The classic form of lead neuropathy is characterized by weakness that initially involves primarily the wrist and finger extensors, but later spreads to other muscles (340).

Sensory involvement is minimal (340). Motor neuropathy is more likely to develop after relatively short-term exposure to high lead concentrations, and evolves in a sub-acute fashion (340). Many ALS-like or ALS cases with antecedent occupational or cosmetic exposure to lead have been documented (324,341-344). A study by Felmus (1976) documented six ALS cases with antecedent occupational exposure to lead for periods of 8-34 years (344). A number of epidemiological studies on long-term exposure to lead have also shown an association with ALS (144,332,336). These findings suggest that prior exposure to lead may be causally associated with the development of ALS.

Lead level in tissues demonstrate a dose dependent increase in the risk of ALS

Demonstrating a dose-dependent relationship between exposure to lead and ALS is important in establishing causality. An increasing positive relationship between lead exposure and the risk of ALS has been shown in certain epidemiological studies (141,326). Nonetheless, the reliability and validity of the exposure ascertainment would be enhanced if supported by biomarkers of lead exposure measured in body tissues or fluids. A dose-response relationship was reported by Kamel et al (2002) in a case-control study of 108 ALS cases in the Boston area, based on measured blood lead concentrations (130,186). In addition, higher levels of lead have been reported in muscle tissue (345,346), cerebro-spinal fluid (CSF), blood, and plasma/serum (161,346-349) in ALS patients, as compared to controls (326,350-355). However, less than 30 subjects were included in most of these studies (161,347-350,355), limiting their power to identify an increased risk of ALS. A report published in 2013 (349) showed that CSF lead concentrations were higher than in blood, suggesting a net influx of lead into the CSF from blood (349). Collectively, these studies are compatible with the findings of the present meta-analysis.

Potential mechanisms whereby chronic exposure to lead might cause ALS

Chronic exposure to lead could cause damage to the renal, nervous, reproductive, endocrine, and immune systems (356), possibly obfuscating a diagnosis of ALS in light of similar symptoms associated with these other health conditions. Pure motor neuron effects following chronic exposure to lead have been observed in chickens (357), which demonstrated motor neuron disease with characteristics similar to human ALS (357). Lead deposition was identified in both the spinal cord and muscles in chickens exposed to lead. Spinal motor neuron

degeneration (in the anterior horn cells) degeneration, motor axonal loss, and atrophy of muscle tissue was also observed in the chickens exposed to lead. As noted previously, many ALS cases have been diagnosed after chronic exposure to lead (324,341-344). Collectively, these results suggest that chronic exposure to lead could result in ALS in humans, in the absence of symptoms associated with exposure to lead being manifested in non-neuronal tissues.

Nearly 200 mutations in metal-binding superoxide dismutase 1 (SOD1) have been linked to ALS (15,358). Animal experiments found that treatment with lead could increase SOD1 expression of mRNA in mice (359), and decrease SOD1 activity in rats (360), suggesting that lead treatment might influence the normal folding process of SOD1 protein, and potentially cause the accumulation of unfolded or misfolded SOD1 protein, a primary mechanism resulting in the apoptosis of motor neurons (361). As a potential link between lead exposure and ALS, we suggest that lead may trigger misfolding of metal-binding proteins (362), such as SOD1, and induce a productive template for propagation of the misfolded protein (363,364).

Limitations of this study

Our study is subject to several limitations. First, the observation of an increased risk of ALS in relation to prior exposure to lead based on meta-analysis demonstrates only association, not causation. However, the weight of evidence in support of a causal relationship is strengthened when the totality of evidence from clinical case reports, epidemiologic studies, and toxicological studies is considered. Second, the accuracy of the risk estimate may be influenced by the quality of included studies, or the quality of the data in meta-analysis, although it is not significantly different among three estimates in our study. The estimate from articles of high quality is attenuated with lower variation. The estimate from adjusted estimates by sex and age is the lowest estimate with lowest variation. To confirm this trend, more observational studies are required. Third, because the present meta-analyses included just two cohort studies, the possibility of recall bias in each of the included studies in meta-analysis cannot be excluded. Four, there remains considerable uncertainty about what fraction of the ALS burden may be caused by lead intoxication. Based on the observational studies available for inclusion on the

present meta-analysis, we estimated that lead could account for up to 5% of all ALS: this could be somewhat of an overestimate, because excess intake of lead is associated with a number of adverse outcomes due to the toxic to almost every organ in addition to the impact on nervous system, including the possible development of ALS (365). However, this estimate was calculated based on an assumption lead causes only ALS.

Conclusion

The results of the present meta-analysis of eight case-control studies suggest that previous exposure to lead in the occupational environment is a risk factor for ALS. Lead might not account for a large number of ALS cases at the present time, since lead pollution has been significantly reduced over last three decades, and lead containing products (such as cosmetics) have been more stringently regulated. Confirmation of the present findings in future studies would serve both to elucidate the causes of ALS, and support risk mitigation actions to further reduce risk of ALS due to exposure to lead from occupational and other sources.

Figures and legends

Figure 1

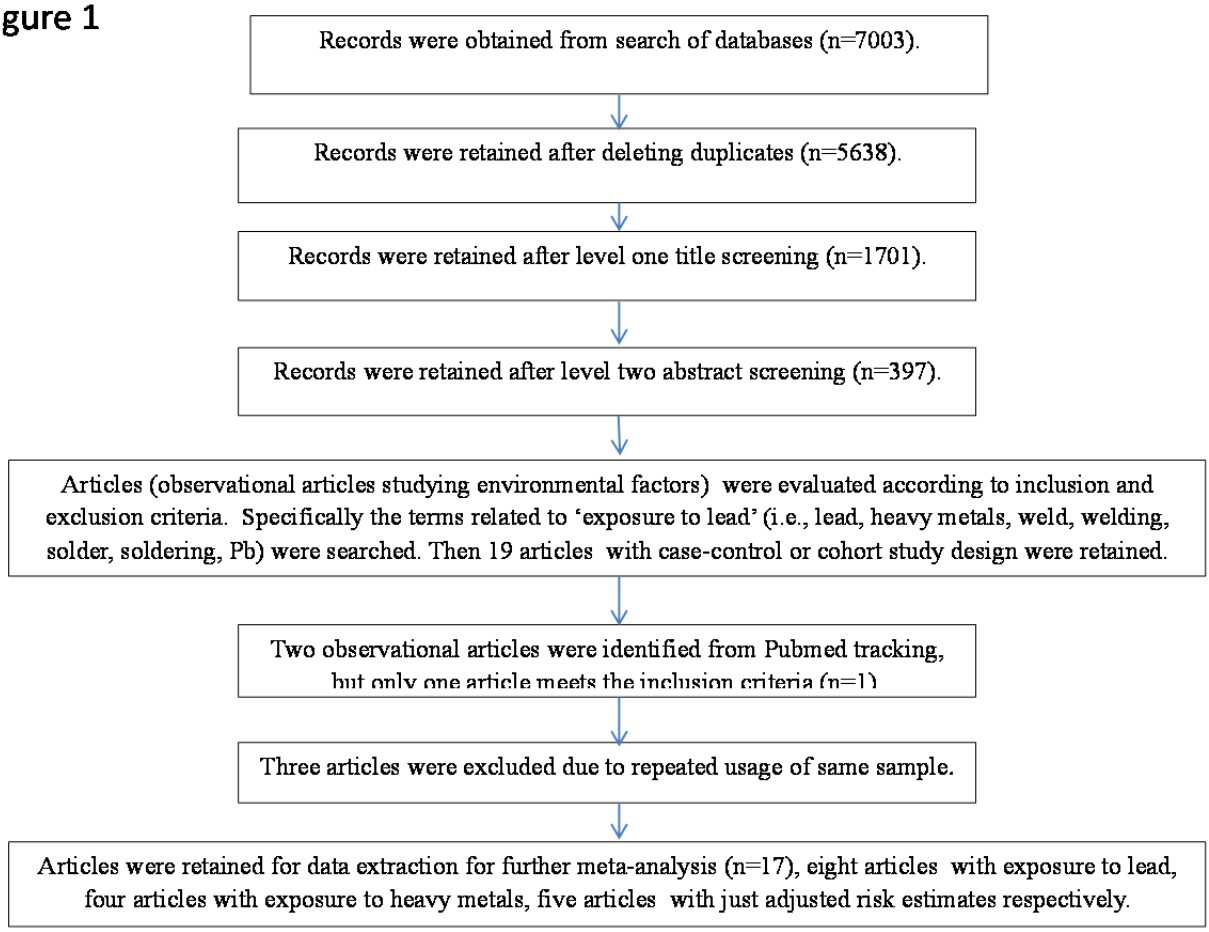

Figure 1. Literature search, screening, evaluation, data extraction, data analysis flow chart for the meta-analysis of observational studies of the association between previous exposure and ALS (25).

Figure 2

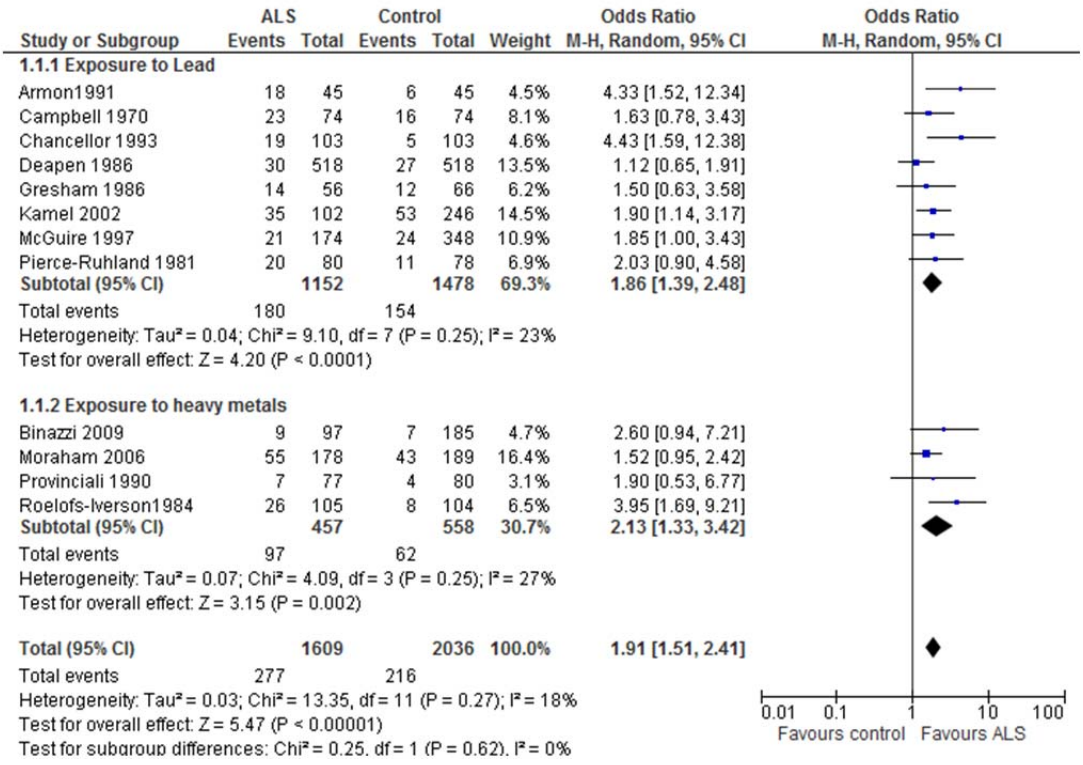

Figure 2. Previous exposure to lead/heavy metals is associated with increased risk of developing ALS. Exposure data were extracted from twelve case-control studies in which exposure to lead (8 studies) or heavy metals (4 studies) and the risk of ALS was assessed (Figure 2). No evidence of heterogeneity across the included studies was observed in a meta-analysis using random effects model. Nor was their evidence of significant publication bias (data not shown).

Figure 3

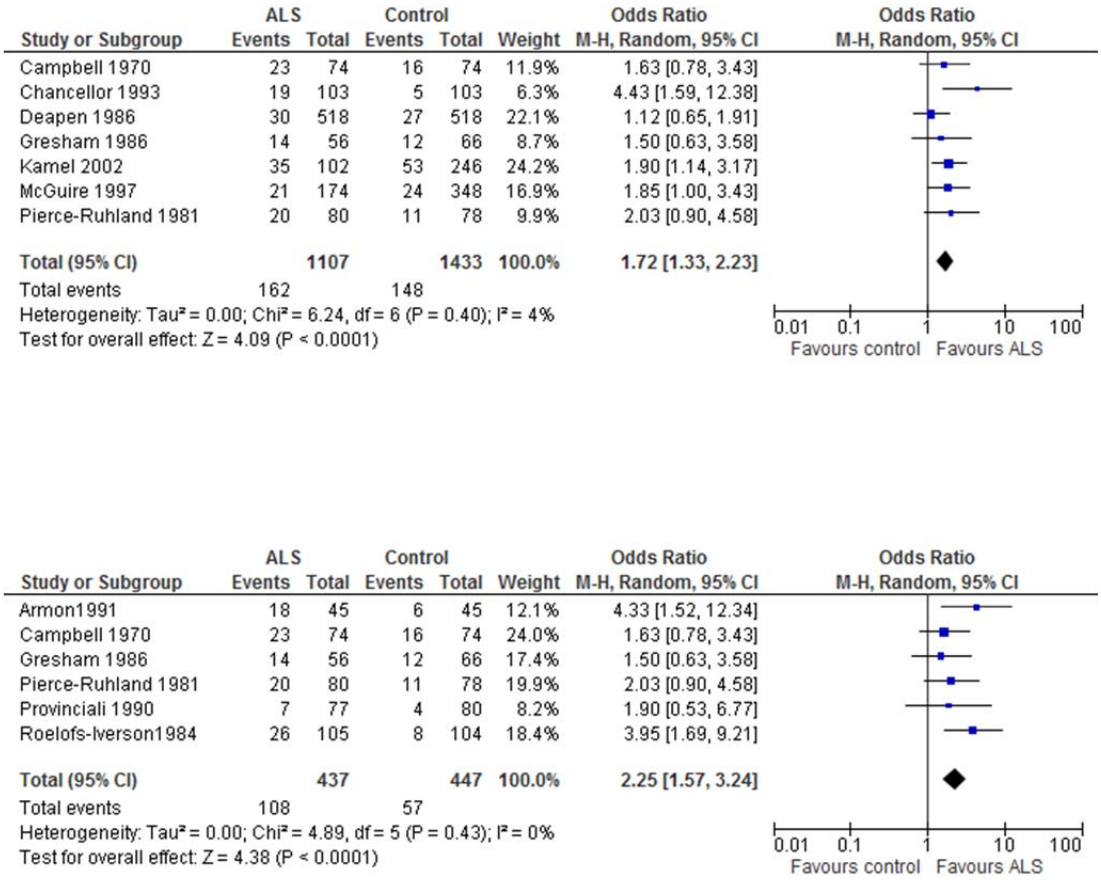

Figure 3. Article quality among included studies does not affect the risk estimate. Included studies were assessed and divided into two groups (six articles each group) based on quality scores. If two articles with identical quality score need to be divided into two groups, then newer publication was allocated into higher score group. The meta-analysis of articles (random effects model) with higher score was showed on top panel, whereas the meta-analysis of articles with lower quality score was showed on lower panel.

Figure 4

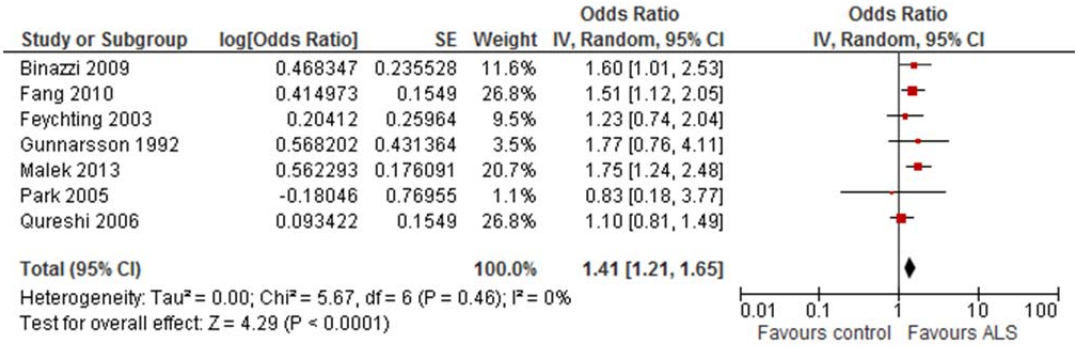

Figure 4. Meta-analysis using adjusted relative risks provided by included articles. The relative risk (OR or RR) from 7 articles were first transformed to log value, then estimated the confidence error based on confidence interval log (95% upper CL -95% lower CL). The meta-analysis was conducted with revman 5.

Key words: Amyotrophic literal sclerosis, exposure to lead, attributable risk, meta-analysis

Acknowledgements: The authors would like to thank Lindsey Sikora, Mona Hersi, and Pauline Quach for helpful advice on the development of the bibliographic search strategy used in this study.

Supplemental document 1

This is a common search strategy for systematic reviews of 'exposure to lead' and ALS from databases medline, psycinfo, and embase. For search of other databases, terms needs to adjust. For review articles related to a specific risk factor, just change step 10. For search of observational studies related to 'exposure to lead', then just skip step 1 to step 6. If search all risk factors to ALS, skip step 1 to step 6, replace step 10 with terms (risk-factor or risk or aetiology or environment* or occupation* or farm* or rural or workplace or toxin or toxic* or infecti* or virus* or bacteri* or industr* or chemical* or agricultur* or pesticide* or insecticide* or herbicide* or fertilizer* or organophosphorus or metal* or trance-element* or lead or mercury or magnesium or aluminum or manganese or selenium or zinc or copper or cadmium or life-style or cholesterol or saturated-fatty-acid or unsaturated-fatty-acid or fat or obesity or overweight or smok* or cigarette* or tabaco* or drinking or alcohol or coffee or tea or vitamin or mineral or physic* or sport* or soccer or baseball or basketball or cyclist or electromagnetic or magnetic-field or solvent* or trauma or injury or genetic* or polymorphism* or SNP or variant* or incidence or prevalence or prognos* or survival).

1. Meta-Analysis/

2. (meta anal* or metaanal*).ab,sh,ti.

3. (methodol* or systematic* or quantativ*).ab,sh,ti.

4. (((methodol* or systematic* or quantativ) adj review*) or overview* or survey*).ab,sh,ti.

5. review.pt,sh.

6. 1 or 2 or 3 or 4 or 5

7. amyotrophic lateral sclerosis/ or motor neuron disease/ or Lou Gehrig's disease/

8. (als or mnd or motor neuron* disease or motor neuropath*).mp.

9. 6 or 7

10. 6 and 9

11. (lead, heavy metals, welding, soldering).mp.

12. 10 and 11

13. limit 12 to humans

14. limit 13 to (english or french)

Supplemental Table1: Summary of included articles related to lead exposure.

Author, Year, Country	Study type	Participant recruitment time, case ascertainment, and study period	Characteristics of population	Risk factor exposure, and risk estimate for als	Reference
Exposure to lead					
Armon C, 1991, US	Case-control	1.Did not mention the recruitment period. 2. Controls were sex and aged matched, one ALS patient selected up to four controls. 3. Excluded familial ALS. 4. Chose ALS patients diagnosed by neurologists only. 5. Other types of MND were also excluded.	1. There were 74 patients with ALS (47 men, 27 women) and 201 controls (100 men, 101 women). 2. Age at interview was 27 to 83 years for patients and 29 to 85 for controls. 3. The average age at first symptom and at diagnosis for the 74 patients with ALS was 59.4 years and 60.8 years, respectively. The median time to diagnosis was 12 months.	Exposure to lead: This was determined and quantified for both members of 45 male ALS patient-control pairs; in 20 pairs **(44%),** at least one member had been exposed. Of these, both members had been exposed in four pairs and one only had been exposed in 16 pairs; the exposed member was a patient with ALS in 14 of these 16 pairs. 2. There was no difference between patients and controls with regard to lifetime trauma.	(366)
Campbell AMG, 1970, UK	Case-control	1. Seventy-four patients attending two centres (Bristol and Bath) after June 1965 in whom a diagnosis of motor neurone disease had been made were seen by one of us (E.R.W.). 2. A control series, matched for age and sex, was obtained from consecutive admissions to a general medical ward, and these patients were interviewed in an identical manner.	1. 74 cases and matched 74 controls by sex and age. 2. 51 males and 23 females of group (control and case). 3. Average age for both groups was 56.8 and 56.2 respectively.	Exposure to lead: 1. 11 and 12 cases were classed into severe exposure (lead poisoning) and light contact (history only) to lead, while 4 and 12 controls were classed into severe and light exposure to lead respectively. 2. A full clinical, occupational, and environmental history obtained by means of a standardized questionnaire.	(326)
Chancellor AM, 1993, England	Case control	1. Patients with incident MND were identified from the Scottish Motor Neuron Register after 1 January 1989 and employs multiple sources of case ascertainment and standardised diagnostic criteria. 147 sALS patients diagnosed between 1 May 1990 and 31 October 1991, 39 had died before approach was possible. 2. Controls were selected by sex, age, living placed matched	The age and sex of cases and controls were identical, a result of the matching process. There were 61 men (mean age 63, range 35-85 years) and 42 women (mean age 67 range 28-86 years).	Exposure to lead: 1. Questionnaire for occupational exposure: a period of regular contact over 12 months or more was recorded as positive. 2. Paired case-control: case/control 2; case only 17; control only 3; neither 81. OR=5.7(1.6-30) Cases (exposed/unexposed)=19/84, Controls(exposed/unexposed)=5/98.	(367)
Deapen DM, Am J of Epi., 1986, USA	Case control	1. Across USA by mailing to all patients who voluntarily contacting to ALS society of USA or clinicians in private or public clinics to distribute the questionnaires to patients with the disease since August 1977. By March of 1977, 1643 patients returned the questionnaires. The 792 patients still living were mailed to a new questionnaires to collect the information about the risk factor, and provide a list of potential controls who were acquaintances to the patients prior to the diagnosis, and at same gender and age (with 5 years).	1. 518 cases, 518 controls 2. 65% are males, 98% are white, 53.3 years old at average. 3. Similar to the remaining 1125 patients (61%, 96%, and 56.1 years respectively)	Exposure to lead: 1. Questionnaire for occupational exposure : Participants were simply asked whether they had ever worked in the presence of selected toxins 2. Exposed to lead (exposed/unexposed): cases=30/488, controls=27/491, OR=1.1(0.6-1.9)	(327)

		2. of the 792 patients, 678 completed the questionnaire. For control, of the 678 contacted, 518 returned the questionnaires.			
Gresham L, 1987, USA	Case control	1. Identified 76 ALS cases, 66 cases participated. 2. Cases were ascertained primarily through a neurology support and research clinic. 3. Identified patients' neighbour/friends: 66, all participated 4. 50% were men in both groups. 5. Recruited from 01/1985-05/1985	1. Mean age (year, SD, range): 59(sd=11.70, 26-84) in cases, 61.5(sd=11.40, 31-83) in controls. 2. 97% were white in both groups 3. There was no difference with regard to twin status, military service, marital status, education levels. 4. Did not mention smoking and alcohol use, life style related factors.	Exposure to Lead, or mercury: 1. The self-administered questionnaire probed potential exposure to nine heavy metals including lead. 2. There was no difference observed with regard to the exposed to occupation related heavy metals. Lead (unexposed/exposed): Cases=52/14, controls=54/12. Mercury (unexposed/exposed): Cases=59/7, controls=60/6. 2. Paired case-control for both lead/mercury: ++=6, ±=13, -/+=6, -/-=8 OR=2.20(0.69-7.47) Lead: 5 12 6 10 2.00(0.63-7.00) Mercury: 2 6 4 21 1.50(0.31-8.26)	(330)
Kamel F, 2002, USA	Case control	1. All study participants (cases and controls) were recruited between 1993 and 1996 2. Cases were required to live in New England for at least 50% of the year, to be mentally competent, and to speak English. 3. 71% of eligible cases participated in the study (n = 111). About 85% of the cases were enrolled within 1 year after diagnosis and the remainder within 2 years. 4. Population controls were identified through random telephone screening, no neurological disease, sex, age (30–55, 56–65, and 66–80 years) and region matched. 354 eligible controls were contacted, 270 (76%) were enrolled, 256 completed the entire questionnaire.	No difference between two groups with regard to the age, sex ratio, education level.	Exposure to lead: 1. Occupational exposure to lead (days of life time) categorized into four levels (0, 1–399,400–1999, and 2000). This lead exposure was cross examined by occupational history, and blood level of lead. 2. ALS risk was increased by exposed to lead. Lead exposure via occupation (unexp/exp): cases=67/35, controls=193/54, OR=1.9(1.1-3.3). 3. Linear dose dependent increase was observed. exp=0, OR=1; exp=1-399, OR=1.6; exp=400-1999, OR=1.9; exp>=2000, OR=2.3, p trend=0.02. 4. Blood lead level was higher in ALS patients (M, SD): Cases=5.2(0.4), controls=3.4(0.4) ug/dl(P<0.05). The increased blood level of lead was associated with an increased ALS risk (up to 10 fold per ug lead increase).	(186)
McGuire , 1997, USA	Case control	1.Recruited during a 4-year period 1990-1994 2. ALS cases aged l8 or elder years who were newly diagnosed with ALS in western Washington State were identified through a surveillance system, but the cases who lacked a telephone or did not speak English were excluded. 3. 180 cases were eligible, 174 cases agreed to participate. 4.Two controls matched to each case according to sex and age (±5 years) were identified from the study counties using random telephone dialling, or for controls over 65 years of age, Medicare eligibility lists for the target counties was used. Overall response rate is 75%.	1. Cases=174, controls=348 2. Men: cases=95, controls=190, account for 54.6% in both group 3. White: case=164, control=329, account for 94.3, 94.5% respectively 4. Age range not difference, but did not provided average age for both groups 5. Marital status was not different 6. Education level might be different significantly (low/high): cases=83/91 cases, controls=124/224 persons.	Exposure to lead -heavy metals: 1.0: Occupational exposure were estimated by selfreported and expert estimated. 1.1. Exp. to lead (self-reported, both sexes, exp/unexp): cases=21/153, CTL=24/324, OR=1.9(1.0-3.6). 1.2. Exp. to lead (panel-estimated, both sexes, exp/unexp): cases=17/157, CTL=31/317, OR=1.1(0.6-2.1). 2. Exposed to heavy metals: 2.1. Self-reported, both sexes, exp/unexp): Cases=84/87, CTL=139/209, OR=1.6(1.0-2.5). 2.2. Panel-estimated, both sexes, exp/unexp): cases=49/124, CTL=82/266, OR=1.2(0.8-1.9). 2.3. Self-reported, males, exp/unexp): cases=63/31, CTL=117/73, OR=1.2(0.7-2.1).	(141)

				2.4. panel-estimated, males, exp/unexp): cases=45/49, CTL=67/123, OR=1.5(0.9-2.6). 2.5. Self-reported, females, exp/unexp): cases=21/56, CTL=22/138, OR=2.3(1.1-4.7). 2.6. Panel-estimated, females, exp/unexp): cases=4/75, CTL=15/143, OR=0.5(0.2-1.4).	
Pierce-Ruhland, 1981, USA	Case control	1. 88 living patients were identified from hospital records. 80 patients participated the study. 2. Patients friends, same sex, age matched with 5 years were suggested by the patients. 80 controls were contacted, 78 controls participated.	1. 80 cases: 53 men, 27 women 2. 78 controls: 52 men, 26 women. 3. Mean age at interview for both groups were the same--52 years. 6. No race data.	Exposure to lead-heavy metals 1. Questionnaire for occupational exposure: 2. Lead (unexposed/exposed): cases=60/20, controls=67/11, OR=2.03(1.22-2.84),p=0.085 3. lead + mercury(unexposed/exposed): cases=51/29, controls=77/11 OR=3.98(3.20-4.76) p=0.001	(167)
Exposure to heavy metals					
Binazzi A, 2009; Italy	Case control	1. Recruited since 17July, 2005 to July 6, 2007. 77 patients, 70 definite, 7 probable. 44 spinal/ 32 bulbar. 2. Diagnosed based on EI Escorial criteria 2. Relatives or accompanying persons of outpatients affected by neurological disease other than ALS, and coming to the same hospital ambulatories, service as population control 3. No response rate was mentioned	1. Cases, 77; 43, males, 34 females, 44 spinal, 32 bulbar. age=65±9.3, range 42-83. onset age: 62.4(60.1-64.7), age at diagnosis, 63.7(61.4-66.1). 2. Control: 185 (male=69, females=116), different to cases. Average=57.5±13.0, 28-84.	Exposure to heavy metals: 1. History of occupational exposure to heavy metals and metal fumes was identified by interview. 2. Exposed to metals or metal fumes, spinal + bulbar controls=7/178, cases=9/68, OR=1.83(0.82-4.14) 3. Exposed to metals or metal fumes, spinal, controls=7/178, cases=8/36, OR=2.94(1.20-7.21)	(333)
Morahan, 2006, Australia	Case control	1. 179 SALS cases. Did not mention when and how they were selected. Cases have donated NDA samples to Australian MND banks, were recruited by MND association. More than 90% ALS patients national wide have been recruited into this bank. 2. No response rate was mentioned. 2. Control: Age ethnicity and sex matched normal subjects with non-neurological diseases. 141 unrelated to patients, 38 related patients. Among unrelated control, 96 were spouses, 35 community controls, 10 acquaintances.	1. Cases: 125 males, 54 females, age, M=60, SD=10 2. Controls: 125 males, 54 females, age, M=61, SD=10	Exposure to heavy metal: 1. Self-reported questionnaires 1. Both sexes (exposed/unexposed):cases=55/123, ctl=43/136, OR=1.41(0.89-2.25) 2. Men (exposed/unexposed):cases=51/74, ctl=41/84, OR=1.41(0.86-2.36) 3. Women (exposed/unexposed):cases=4/49, ctl=2/52, OR=2.12(0.43-10.45)	(146)
Provincia li L, 1990, Italy	Case control	1. 77 patients (57 males, 20 females, mean age=59±8) from 89 consecutive patients from a clinic during 1979-1987. 2. Controls (80) with various types of neuron diseases(including infectious diseases)were matched by age, sex, life-style(alcohol drinking, smoking), education, origin, cultural background from in patients over same period	no race information.	Exposure to heavy metals-hard labor 1. Hard labor(exposed): patients=54, control=39, RR=2.4, Chi-sqaure=6.4, P<0.05 2. Exposed to heavy metal: patients= 7. Control= 4, RR=1.8; Chi-square= 1.0.	(334)
Roelofs-Iverson RA,	Case-control	1. Cases were diagnosed by neurologists recruited from 1978 to 1979. 2. Of the 145 patients who received the	The average age at onset was 55.6 years, with range from 19 to 83 years. There were 47 women (average age of onset, 57.6) and 58 men	Exposure to heavy metals: Of the 105 ALS cases, 26 reported exposure to one or more of these heavy metals. Of the controls, 8 of 164	(368)

1982, USA.		questionnaires, 105 replies were included. 3. Included 6 familial ALS patients. 4. Controls were not described.		were exposed**.**	
Meta-analysis with adjusted OR or RR.					
Feychting M, 2003, Sweden	Cohort Study	1. All individuals included in the Swedish census in 1980 who were economically active in 1970 or in 1980, and who were alive on 1 January 1981, for a total of 4,812,646 subjects (2,649,300 men and 2,163,346 women). We followed the subjects from 1 January 1981 until 31 December 1995 or until death, whichever came first.	1.The age range at the start of follow-up was 16 to 98 years, with a median of 43 years Cases average-age cases average-age Alzheimer's disease (331.0) 1321 77 679 76 Vascular dementia (290.4) 2064 80 604 81 Senile dementia (290.0) 8017 81 3736 82 Presenile dementia (290.1) 1336 74 642 74 ALS (335.2) 1411 70 554 69 Parkinson's disease (332.0) 5136 78 1153 78 Multiple sclerosis (340) 521 61 274 57 Epilepsy (345.1-245.9) 1244 66 359 70	Exposure heavy metals: Welders (1.12 T): cases=24, RR=1.6(1.1–2.4)	(335)
Gunnarsson, 1992, Sweden	Case control	1. Cases in the age range 45-79 and to a random sample of 500 population controls in the same age range. The questionnaires were answered by 92 cases and 372 controls, a response rate of 85% and 75% respectively. 2. The study population was limited to those aged 45-79 and consisted of 1-2 million inhabitants. 500 controls represented this population. 3. Total of 112 patients diagnosed with MND in the age range 45-79 was identified in the study area.	1. Completed questionnaires were received from 92 cases of MND (58 men and 34 women) and 372 controls (189 men and 183 women), corresponding to a response rate of 85% among the cases (men 83% and women 89%) and 75% among the controls (men 74%o and woman 75%). 2. Among the controls the proportion of respondents was proportionately low among those working in agriculture or forestry (63%) but among the cases, this proportion was equal to other occupations. The response rate between the different counties ranged from 61 to 83 % for the controls and from 74 to 100% for the cases.	Exposure to heavy metals Welding-Lead vapor welding MHOR = 3.7 (1.1-13.0)	(147)
Malek A, 2013, USA	Case-control	1. SALS patients were recruited from 3 major medical neurology clinics with ALS centers, 2 in Pittsburgh, Pa., and 1 in Philadelphia, Pa., between December 2008 and July 2010. 2. Diagnosed with EI Escorial. 3. FALS cases, and ALS cases with other neurological conditions were excluded. 4. Cases and controls were required to speak English. Controls were selected from the corresponding geographic region (W. Pa. or Greater Philadelphia area) and 1: 1 matched to cases by age of onset/first ALS symptoms (±5 years), sex and race. 5. Of the 106 patients contacted about the study, 78 participated, with a response rate of 73.6%. But only 66 cases were used for analysis due to lack of the corresponding controls 6. Occupations held for at least 2 years since the age of 19 years	The majority of cases and controls were male (68.2%), Caucasian/White (98.5%) and from W. Pa. (86.4%). The cases (mean ± SD: 57.1 ± 13.2 years) were slightly older than the controls (56.4 ± 13.5 years), with a trend for older cases (p = 0.07).	1. Those with occupational exposure to pesticides potentially had 3.17 times the risk of developing ALS compared with those without exposure (OR = 3.17; 95% CI:1.27, 7.93). 2. Occupational exposure to electrical and electromagnetic equipment and machinery and electromagnetic fields appeared protective for cases (OR = 0.41; 95% CI: 0.20, 0.82). 3. Exposure to heavy metals: 19/66, OR=0.89 (0.84-4.24) 4. After controlling for smoking (ever vs. never) and education (high school or less vs. more than high school), cases reported significantly greater occupational exposure to metals (OR =3.65; 95% CI: 1.15, 11.60) and pesticides (OR = 6.50; 95% CI: 1.78, 23.77) than controls. Occupational exposure to electrical or electronic equipment, machinery or electromagnetic fields again appeared protective for cases (OR =0.28; 95% CI: 0.11, 0.69).	(152)

				5. Additional individual risk fact was also conducted, but data not shown, because sample size is too small.	
Park RM, 2005, USA	Cohort Study	1. Death certificate information for all deaths from 22 participating states in the years 1992–1998 was obtained using the National Occupational Mortality Surveillance System 2. Count underlying and contributing causes of death. 3. Controls were all decedents with no mention of neurologic disease, degenerative or otherwise, and excluding: accidental causes, malignant neoplasms of the brain (ICD 191), other senile and presenile organic psychotic conditions (ICD 290), diseases of the nervous system and sense organs (ICD 320–389), and finally, neoplasms of the lymphatic and hematopoietic tissues (ICD 200–208), due to suspect associations with solvents or electromagnetic fields (EMFs).	Order: White men White women Non-white men Non-white women Total Motor neuron disease: 3,851 2,152 203 141 6,347 Total deaths: 1,479,921 803,110 203,862 127,453 2,614,346	Exposure to heavy metals: Welding Occupations-manganese fumes (no lead?) total death ALS 1. Deaths, n 44,545 70 MORa(95% CI) 0.66(0.49-0.88) (adjusted estimate)	(150)
Qureshi MM, 2006,USA	Case control	1. Between April 1998 and August 2002, recruited 95 subjects with ALS and 106 healthy control subjects in this study. 2. Cases were identified from clinic. 3. Controls were non-blood relative (spouse), friends, unrelated subjects(age matched).At recruitment controls were matched to the ALS subjects by gender and age.	Cases Controls Gender – Males 60 (63.2 %) 58 (54.7%) Race – Caucasian 91 (95.8 %) 102 (96.2 %) Mean age at enrol.:54.4 ±13.1 (SD) 52.5 ±14.9 (SD) Weight (Kg) – F: 66 ±19 (SD) 65 ±10 (SD) Weight (Kg) – M: 82 ± 13 (SD) 86 ± 17 (SD)	Exposure to lead-heavy metals 1. Of the 95 subjects in the ALS group, the toxin exposure most commonly reported was exposure to pesticides (n=18), followed by lead (n=15), industrial solvents (n=5), mercury (n=5) and miscellaneous toxins. 2. Welding as occupation 1.24 (0.54–2.87), p= 0.61	(144)
Samples were duplicate used					
Fang F, 2009, USA	Case control	See other reports from same authors (Kamel)	1. The median age at diagnosis for cases was 60 years (range, 30–79 years) and the median age at 2 years before interview for controls was 59 years (range, 29–78 years). Diagnosis was made 14.3 months after the onset of symptoms on average. 2. Male: Cases, 66 (60.6%); controls, 156 (61.7%). Female: Cases, 43 (39.4%); controls, 97 (38.3%) 3. 27 (25%) of the cases had a bulbar onset and 82 (75%) had a trunk or limb onset.	Exposure to lead: Exposed in cases or controls. Overall: 55 35 1.9(1.1–3.4) ; Smokers 33 25 1.9(0.9–3.8); Non-smokers: 22 10 2.8 (1.0–7.8) . Ccases(exposed/unexposed)= 35/84, Controls(exposed/unexposed)=55/201.	(143)
Fang F, 2010, USA	Case control	1. VALE included a subset of registry cases comprising motor neuron disease cases who donated a blood sample between January 23, 2007, and September 30, 2007; 208 cases were eligible, of whom 200 were enrolled, including 163 ALS cases (including 12 possible cases), 30 progressive muscular atrophy cases, and 7 primary lateral sclerosis cases. Most studies only use definite and probable cases.	1. 2003–2007 2. 184 cases and 194 controls among US veterans, most are whites, 94% in cases, 98% in controls. 3. 98% are males in cases, 94% in controls	Exposure to lead 1. After adjustment for age, a 1-unit increment of log2-transformed lead (equivalent to a doubling of blood lead) was associated with a 2.6-fold higher odds of ALS (95% confidence interval: 1.9, 3.7). Adjustment for smoking (ever/never) in addition to age did not change the results. 2.A dose response for the lead-ALS association was also seen when blood lead was categorized in tertiles;	(130)

		2.Between May 2007 and May 2008, VALE contacted 359 controls already enrolled in GENEVA for additional informed consent and blood sample collection. A total of 252 controls consented to participate in VALE, of whom 229 ultimately donated a blood sample. 3. Response rate: cases, 200/208; controls, 229/252.		after adjustment for age and CTX, the odds ratio for the highest compared with the lowest tertile was 2.1 (95% confidence interval: 1.1, 3.8; P trend= 0.008).	
Kamel, 2005, USA	Case control	1. All study participants (cases and controls) were recruited between 1993 and 1996 2. Sequential ALS cases were recruited from two major referral centers in New England. 3. Cases were required to live in New England for at least 50% of the year, to be mentally competent, and to speak English. 71% of eligible cases participated in the study (n = 111). About 85% of the cases were enrolled within 1 year after diagnosis and the remainder within 2 years. 4. Population controls were identified through random telephone screening, no neurological disease, sex, age (30–55, 56–65, and 66–80 years) and region matched. 354 eligible controls were contacted, 270 (76%) were enrolled, 256 completed the entire questionnaire.	See another report from same authors because they used same data set.	Exposure to lead 1. Ever exposure to lead (unexposed/exposed): cases=67/35 controls= 193/53 OR=1.9(1.4-2.6) 2. gradient of lead(number, p trend=0.02): 0 67 193 1-399 8 17 1.6(0.6-3.9) 400-1999 11 17 1.9(0.8-4.3) >2000 16 20 2.3(1.1-4.9) 3.Lead blood level(ug/dl): cases=5.2±0.3; control=3.4±0.4 (p<0.05)	(332)

Chapter 5. Previous exposure to agricultural chemicals is associated with an increased risk of developing ALS–Evidence from meta-analysis of observational studies

Ming-Dong Wang[1], Julian Little[1], James Gomes[1], Neil Cashman[2], and Daniel Krewski[1]

1. Department of Epidemiology and Community Medicine, Faculty of Medicine, University of Ottawa, Ontario, Canada, K1H 8M5

2. Department of Medicine, University of British Columbia, Vancouver, BC, Canada V5Z 1M9

Abstract

Amyotrophic lateral sclerosis (ALS) is a rare terminal degenerative condition of motor neurons. Its causes are still largely unknown, except for a small proportion of ALS cases linked to multiple monogenic mutations. Previous exposure to agricultural chemicals including pesticides has been linked to the development of ALS in observational epidemiological studies. To estimate the risk of developing ALS after exposure to pesticides, we adopted a systematic review and meta-analysis approach to synthesize the information from existing observational studies searched through to January, 2014 based on PRISMA guidelines. The meta-analysis revealed that, based on 9 studies including 307 cases of ALS with exposure to pesticides among a total of 1,891 ALS cases, exposure to pesticides was associated with an increased risk of ALS [OR=1.53(95% CI: 1.18-1.99) under a random effects model]. The maximum attributable risk of sporadic ALS due to previous exposure to pesticides was estimated to be 5.5% of all ALS cases. These results suggest that previous exposure to pesticides may be a risk factor for ALS, although such exposures may account for only a small fraction of all ALS cases.

Introduction

Amyotrophic lateral sclerosis (ALS), also known as Lou Gehrig's disease in North America, is the most common form of motor neuron diseases (MND), characterized by rapidly irreversible progression of weakness of voluntary muscles (38), beginning in the limbs (60-80%), or bulbar musculature (20-40%) (40,93). A small proportion (~1%) of ALS with an atypical disease course has been diagnosed with respiratory failure as the initial symptoms (369). ALS is usually considered an adult onset neurological condition, presenting either in familial (5-10%) (1) or sporadic forms (>90%). However, juvenile ALS has also been reported, again presenting in familial or sporadic forms (370), but caused by monogenic mutations (371-374). Overall, the incidence of ALS is low, about 2-3 cases per 100,000 persons per year (45,46) worldwide. Although ALS affects males more frequently than females (55), the biological basis for this difference has not been elucidated. Only one approved drug (riluzole) is available for the treatment of ALS, and it appears to prolong life by only a few months (375). Hence, there is considerable value in understanding aetiology with a view to identifying possible strategies for primary or secondary prevention.

Since there is currently no specific biological biomarker for early diagnosis of ALS, the diagnosis of ALS is dependent on the exclusion of other possibilities and differentiation from other degenerative neuron diseases (38). The time from the appearance of first clinical symptoms to diagnosis is usually about a year (376). Newly amended diagnostic criteria for ALS (the Awaji criteria) permits slightly earlier diagnosis (377). ALS patients usually die within 2-3 years of diagnosis (90), mainly due to failure of respiratory function (2,378-380). However, a small proportion (~10%) of ALS patients survives for more than 10 years (381), and even decades in some rare cases (37), reflecting extreme heterogeneity in disease progression (382).

ALS is a rare neurodegenerative condition that occurs worldwide across different ethnic backgrounds. The crude incidence rates of this disease in Europe and North America are very similar, approximately 2-3 cases/100,000 persons / year (45,46). The median reported incidence from European countries is 2.09 cases/100,000 persons/year, and the median prevalence is 5.40 cases/100,000 persons (47). The incidence in Asia is lower (49,92,376), but the highest incidence (50-100 fold above the world average) was seen in Guam and surrounding islands during the

period 1950-1960 (48,50-53). Populations with Spanish or Black backgrounds appear to be somewhat resistant to ALS (94). Although the incidence increases with age, it peaks at 50-70 years of age, and declines rapidly thereafter (46,383). These observations suggest that ALS is not an aging-related neurodegenerative condition (58).

ALS cases are classified into familial and sporadic forms, based on familial aggregation. Familial ALS is determined if at least two ALS cases have been diagnosed from first or second degree relatives in a family (60,62,384). The familial form of ALS accounts for about 5-10% of total ALS cases (36), with the remaining 90-95% of ALS cases occurring sporadically. However, this classification is out-dated in the current era of molecular biology, because a substantial proportion of ALS cases with monogenic mutations is observed in individuals with apparent sporadic disease (3). On the other hand, some familial ALS cases may be associated with environmental factors, a finding indirectly supported by the observation of conjugal cases (104-110).

Monogenic mutations have been identified in about 50% of FALS cases, and about 5% of SALS cases (3). The most common mutated genes are *SOD1* (worldwide) and C*9orf72* (whites). The repeat expansion in C*9orf72* originated from north Scandinavia only a few thousand years ago (7,8). Although more monogenic mutations are being identified among ALS cases, such mutations are responsible for only about 10% of ALS cases (3). It is estimated that genetic risk factors, including polymorphic variants, and environmental risk factors play an equally important role in the development of ALS (17,18). Most ALS cases are sporadic, but their distributions in general population is not random. Temporal (90), geographic (90,92), occupational clustering (93), or affected spouse pairs (104-110) have been reported in almost every country, with these types of clusters often having been reported to be associated with at least one possible environmental risk factor. To date, no single environmental risk factor has been conclusively confirmed and verified to be associated with the occurrence of sporadic ALS cases (385).

A growing body of evidence shows that being involved in agricultural activities, or having experienced previous exposure to agricultural chemicals, is a risk factor for sporadic ALS (123,139,140,154). Living in a rural area has not been shown to be a risk factor for ALS

(120,123,144,149). The association between exposure to pesticides and the risk of ALS was also reported in a small scale prospective study in which the mortality due to ALS in a pesticide factory was 3.4 times higher than expected ($P<0.05$) (386). In addition, there are a number of case reports of ALS with antecedent exposure to pesticides (387-391). To explore the association between previous exposure to pesticides and ALS, we conducted a serious of meta-analyses using the data extracted from observational studies, following current guidelines for systematic review and meta-analysis of existing data (25).

Method and Materials

The PRISMA guidelines have been followed in reporting the results of this systematic review (25). A flow chart summarizing the numbers of articles identified by the search strategy and the different steps of screening, evaluation, data extraction, and analysis is presented in Figure 1.

1. Literature search strategy: Peer reviewed publications including systematic reviews and observational studies related to risk factors of sporadic ALS were collected. The search strategy was first developed within Medline, using search terms describing the disease characteristics and environmental risk factors of interest. Once the strategy was optimized, it was used to search related articles in other databases (PubMed, Embase, CINAHL, Toxline, and Psychinfo). The bibliographies of included articles were also searched to identify relevant articles, especially older publications. New publications were tracked and updated by searching PubMed using the disease terms only. Grey literature was identified by searching the Google Scholar website. The databases were searched through to March 18, 2013.

2. Selection and eligibility criteria for observational studies: A total of 5,638 articles was found in the initial search, after removing duplicates using Reference Manager by comparing adjacent references' authors and titles after sorting references by author name. The articles were screened in DistillerSR using predesigned screening forms. Level one screening was conducted by reading the titles in relation to the inclusion/exclusion criteria [English, human study, disease terms, environmental risk factors, and observational epidemiological study information (cohort,

case-control, or cross-sectional, but not a review, commentary, or intervention study)]. A total of 1,071 articles were retained after removing articles deemed non-relevant by this process. Level two screening was conducted by examining abstracts in relation to the same inclusion criteria as step one. A total of 397 articles was retained for full article review at level three screening by examining the complete article and applying additional inclusion/exclusion criteria [in addition to the criteria mentioned above, population information, case ascertainment by ICD or El Escorial criteria, environmental risk factors (articles dealing with military service, or ALS-PDC only were excluded since former one is not a risk factor for general public, and later one is associated with location specific risk factor), method of data analysis, and results had to have been presented] by two reviewers (MDW and JG). A final list of 88 articles was identified for possible inclusion in relation to environmental risk factors for ALS in this study. At this level of screening, consensus between the two reviewers had to be reached. Of these 88 articles considered, 23 articles relating to pesticides or with occupations likely associated with exposures to pesticides (farmers, farming. countryside, rural, agricultural activity, agricultural chemicals, herbicides, insecticides, fungicides, rodenticides, and pediculicides) were selected (Table 1). Three additional relevant articles were identified after this initial search of multiple databases via tracking PubMed (140,152,392). Since ALS is a rare neurological condition, a key evaluation item is to examine if information on the same sample of patients with ALS has been reported in more than one article. If so, we arbitrarily decided that the earliest article would be retained for meta-analysis. Relevant information was then abstracted from these articles, including information about the study population, study period, study country, response rate, control information, diagnostic criteria, data analysis methods, risk factor information, and main results. A random sample of the studies from which data had been abstracted was evaluated by the second reviewer.

3. Data extraction and analysis: A data extraction form was designed to collect the information from the selected studies. Extracted data were verified by the second reviewer. Meta-analyses were conducted to compare the risk of ALS in farmers or in the individuals with previous exposure to agricultural chemicals to controls. Although synthetic odds ratios were calculated using Reviewer Manager 5 using both random and fixed effects models, we reported the ORs primarily from the random effects models. Heterogeneity across included studies was evaluated using Tau^2, Chi^2 and I^2. Forest plots and relevant supporting statistics were presented in order to

evaluate publication bias in the studies selected for inclusion in our meta-analyses. Sensitivity analyses were conducted based on study types, publication year and quality.

4. Maximum attributable risk (MAR) due to previous exposure to pesticides: To help understand the risk estimates regarding to the previous exposure to pesticides, we estimated the attributable risk due to previous exposure to pesticides among total ALS cases based on case-control studies only. The combined prevalence data for meta-analysis of the association between ALS and exposure to pesticides was used to estimate the maximum attributable risk (MAR) due to previous exposure to pesticides among ALS cases (338). The assumptions underlying this formula are: 1) the disease prevalence in the population is low (usually <5%); 2) previous exposure to pesticides is causally associated with ALS; 3) the ALS cases and controls included in the selected observational studies are representative of the total population of ALS patients and general population; and 4) all excess exposure events among ALS cases as compared to controls are responsible for the development of ALS among those who reported being exposed to pesticides. Under these assumptions, this attributable risk has been called as maximum attributable risk (MAR), calculated as

$$MAR\ (\%) = p(r-1)/[pr + (1-p)]x100\%,$$

where p denotes the proportion of persons exposed to pesticides in the control population; r denotes the relative risk (RR) of ALS associated with previous exposure to pesticides.

Results

1. Literature search summary: The literature search strategy, including article screening and evaluation and data extraction and synthesis is illustrated in Figure 1. Among the 26 articles included in this review, there were 6 cross-sectional, 4 case-control and 1 cohort studies with farming related data, along with 9 case-control and 2 cohort studies with data on previous exposure to pesticides and, more generally, to agricultural chemicals. These articles form the basis of this analysis.

Four studies were excluded from further meta-analyses since they did not provide exposure prevalence data. Among these four studies, one cohort study using mortality data from 1992 to 1998 in 22 US states revealed that the relative risk of developing ALS among adult individuals of all farming related occupations was significantly increased by 20% [MOR (mortality odds ratio, calculated using logistic regression)=1.20, 95% CI: 1.02-1.41] (150). However, the increased mortality may be mainly attributable to individuals aged ≥65 years because the MOR among individuals aged < 65 years was lower and not statistically different (MOR=1.09, 95%CI: 0.78-1.50) (150). This conclusion is consistent with an included cohort study conducted in Sweden that also reported a significant association between agricultural activity and ALS (OR=1.7, 95% CL: 1.1-2.7) in Figure 2 (189). Both cohort studies provided the strongest evidence for the association between agricultural activity and ALS. Two case-control studies using 'rural'(128,393), one case-control study using 'toxin' were also excluded (144).

2. The association between ALS and farming occupation was supported by cohort and case-control studies: The first meta-analysis of the association between ALS and farming activity was conducted with the data from 11 studies (6 cross-sectional, 4 case-control, and 1 cohort) of the relationship between farming and ALS (Figure 2). The results showed a significant disproportion of farmers among ALS patients, compared to corresponding controls under random effects model (OR=2.04, 95% CI: 1.20-3.44). However, the heterogeneity across included studies was significant under either model [random effects model ($P < 0.00001$; $I^2 = 96\%$), fixed effects model ($P < 0.00001$; $I^2 = 96\%$)] (Figure 2, other results not shown).

The subgroup analysis showed that cross-sectional studies contributed significantly to the observed heterogeneity. The heterogeneity within 6 cross-sectional studies is extremely high (Heterogeneity: $P < 0.00001$; $I^2 = 97\%$, random effects model) (Figure 2 top panel). In contrast, the heterogeneity across the included 4 case-control and one cohort studies was modest the (Heterogeneity: $P = 0.16$; $I^2 = 39\%$, random effects model),) (Figure 2, lower panel). The risk of ALS among individuals with agricultural occupations under both models was significantly increased [random effects, OR=1.77, 95%CI: 1.37-2.28) (Figure 2)] in case-control studies.

3. Occupational exposure to pesticides or agricultural chemicals is associated with ALS: Agricultural activity may be only associated with, but not cause, ALS. Exposure to other risk factors such as pesticides during agricultural activity may be responsible for the overrepresentation of farmers among ALS patients.

The subgroup analysis showed that the OR of subjects with history of exposure to pesticides among ALS patients versus among controls was significantly increased [OR=1.53 (1.18-1.99), random effects model] (Figure 3), with no significant heterogeneity detected [random effects model, P = 0.06); I^2 = 46% (Figure 3]. The estimate from two included case-control studies using agricultural term seems higher (OR=3.08, 95% CI: 1.43-6.63), but not significantly different from the estimate based on the exposure to pesticides. Therefore, both articles were excluded from the estimate of attributable risk analysis below. Funnel plots did not show a publication bias (data not shown).

The subgroup analyses were also conducted based on publication years and study quality. The risk estimate (OR=1.47; 95% CI: 0.96-2.25) based on meta-analysis from newer publications (2009 or after) is not different from the estimate (OR=1.77, 95% CI: 1.32-2.37) from older articles (before 2009). We also classified all articles into two categories based on the study quality using criteria developed in our team (23). The risk estimate (OR=1.51, 95% CI: 1.05-2.19) from higher quality articles (140,141,143,148,149,152) is not different from the estimate (OR=1.88; 95%CI: 1.39-2.54) from lower quality articles (123,142,145,146,327) under random effects model.

4. Maximum attributable risk (MAR) due to previous exposure to pesticides: A total of 219 ALS cases from a population of 1,189 individuals with ALS had been exposed to pesticides previously, whereas a total 195 controls from 1,550 normal subjects had been exposed to pesticides based on the studies that formed the basis for the present meta-analysis. To calculate the MAR, we note that the prevalence of exposure to pesticides among controls is given by p= 195/1,550=0.1258 and the relative risk, r=[(219/1189)/(195/1660)]=1.4641. The MAR is then estimated to be 5.5% of total ALS cases, which suggests that up to 65 of the total of 1,189 ALS cases from all included studies might have been be caused by previous exposure to pesticides.

Discussion

The analytic results of this study indicate that ALS is associated with agricultural activity, possibly via previous exposure to pesticides and agricultural chemicals, a finding consistent with two recent meta-analyses (139,140). However, our study not only updates both meta-analyses, but also explores the relationship between agricultural occupation and exposure to pesticides. We estimated that up to 5.5% of ALS cases could be due to exposure to pesticides. Although previous exposure to pesticides should be considered a risk factor for ALS, its contribution to the total burden of ALS is modest, similar to that of other common risk factors such as heavy metal and trauma for ALS (results based on our own unpublished studies).

Rural residence was associated with higher incidence or mortality of ALS in some studies (148,392,394,395), but not in others (124,128,396). Apparently, rural residence itself is not a risk factor for ALS; rather, the true risk factors for ALS are associated with rural residence, i.e., exposure to agricultural chemicals (120,123,144,149). Here we discuss the likelihood of this association**.**

Population based toxicity studies support the association of previous exposure to pesticides with ALS: Organophosphorus (OP) compounds have been used as pesticides/insecticides, fertilizers and warfare nerve agents for centuries. OP compounds specifically inhibit NTE (neuropathy target esterase), an enzyme in cholinergic neurons, and induces delayed polyneuropathy (OPIDP), mainly affecting voluntary muscles (397), mimicking the phenotype of ALS. In 1994, Tosi and his colleagues revisited an outbreak of paralysis that occurred in the fall of 1942 to the following summer in a farm in Italy (37). The paralysed patients were diagnosed with neuropathy but, in retrospect, many of the symptoms/clinic signs were similar to those of ALS. Fifty years later, some patients still showed the same symptoms (37). The authors therefore concluded that the original outbreak was OPIDP. Organophosphate contamination of ground soil and water has been reported in developing countries; and has affected the motor neurons in exposed populations. Goss has described a seasonal epidemic of 'new' syndrome for about 20 years in Northern China (398). This syndrome mainly affected children and young adults in rural areas, with symptoms in the limbs, but not the sensory nerves, originally often misdiagnosed as

Guillain–Barré syndrome (398), which has a phenotype similar to that of ALS. An earlier report from China described 143 cases of pesticide poisoning causing delayed dysneuria, some of which were symptomatically similar to ALS, and often diagnosed as ALS (399).

Case reports of OP exposure appear to support a causal relationship between pesticide exposure and ALS. The first ALS case possibly associated with the exposure to pesticides was reported in 1987. A 59-year old man progressively developed typical ALS symptoms with normal sensory testing after spraying two cans of pesticides in an unventilated room (387). OP insecticides exposure was linked to two cases of chronic motor neuron disease in Brazil in 1993 (388), a case of neuron motor disorder due to chronic exposure to pyrethroid insecticide in France in 2011 (389), a similar case due to chronic exposure to DDT or organophosphate pesticides in Greece (390), and a case due to chronically exposure to pesticides for three years in Japan (391). All these case reports appear to suggest that exposure to pesticides and other agricultural chemicals is a risk factor for the development of sporadic ALS.

Several studies report higher mortality from ALS among sport-persons, as observed among Italian professional soccer players, but not in basketball players and cyclists (180-183). Other researchers have reported that mortality attributable to ALS in professional American football players was higher than expected (184). Using pesticides and other chemicals to treat the soccer and baseball fields was a common practice in the past in Europe and North America. Therefore, professional soccer players, as well as baseball players, were frequently exposed to agricultural chemicals. Despite some controversy (400), studies showed that mortality among Gulf war veterans with ALS was significantly higher than expected (400,401). Since some veterans had received prophylactic treatment containing cholinergic inhibitors to protect against nerve gas (120), suggested that some veterans might have been exposed to OP (402,403). Collectively, these studies strongly suggested that exposure to pesticides is a risk factor for ALS.

Potential mechanisms: Although many epidemiological studies have linked the development of the previous exposure to ALS, however, the biological plausible mechanisms involved have not understood. The potential mechanisms include modulating gene expression via epigenetic modification (histone modification and DNA methylation), impairing mitochondrial function by

perturbing ATP production, and inducing oxidative stress by stimulating the production of cellular oxidative species{5072 Mostafalou,S. 2013}}. The common pathological change in ALS is the accumulation of unfolded proteins, which might act like prion-like proteins to trigger propagation of the misfolded form (363,364), eventually leading to apoptosis of affected motor neurons. Pesticides may increase the accumulation of misfolded proteins by stimulating protein oxidation (404,405).

Conclusion and limitation: Taken together, the available epidemiological evidence suggests that previous exposure to pesticides is associated with ALS. This relationship may explain the association between agricultural occupation or living in rural areas and the development of ALS. Many anecdotal case-reports also suggest that the signs and symptoms observed among those exposed to pesticides are quite similar to those observed among ALS patients. The phenotypes of neurotoxicity observed among pesticide-exposed individuals and the neurotoxicity exhibited by ALS patients appears to be very similar. Therefore, it appears that previous exposure to neurotoxic pesticides is a risk factor for ALS. However, this conclusion, which is based on meta-analysis of observational data, needs support from further studies designed to elucidate the biological mechanisms of action suggested above. Even if previous exposure to pesticides is a risk factor for ALS, the estimated 5% maximum relative attributable risk may be an overestimate. . Pesticide exposure may be responsible for a number of adverse health outcomes (64), of which ALS comprises a very small proportion.

Figures and legends

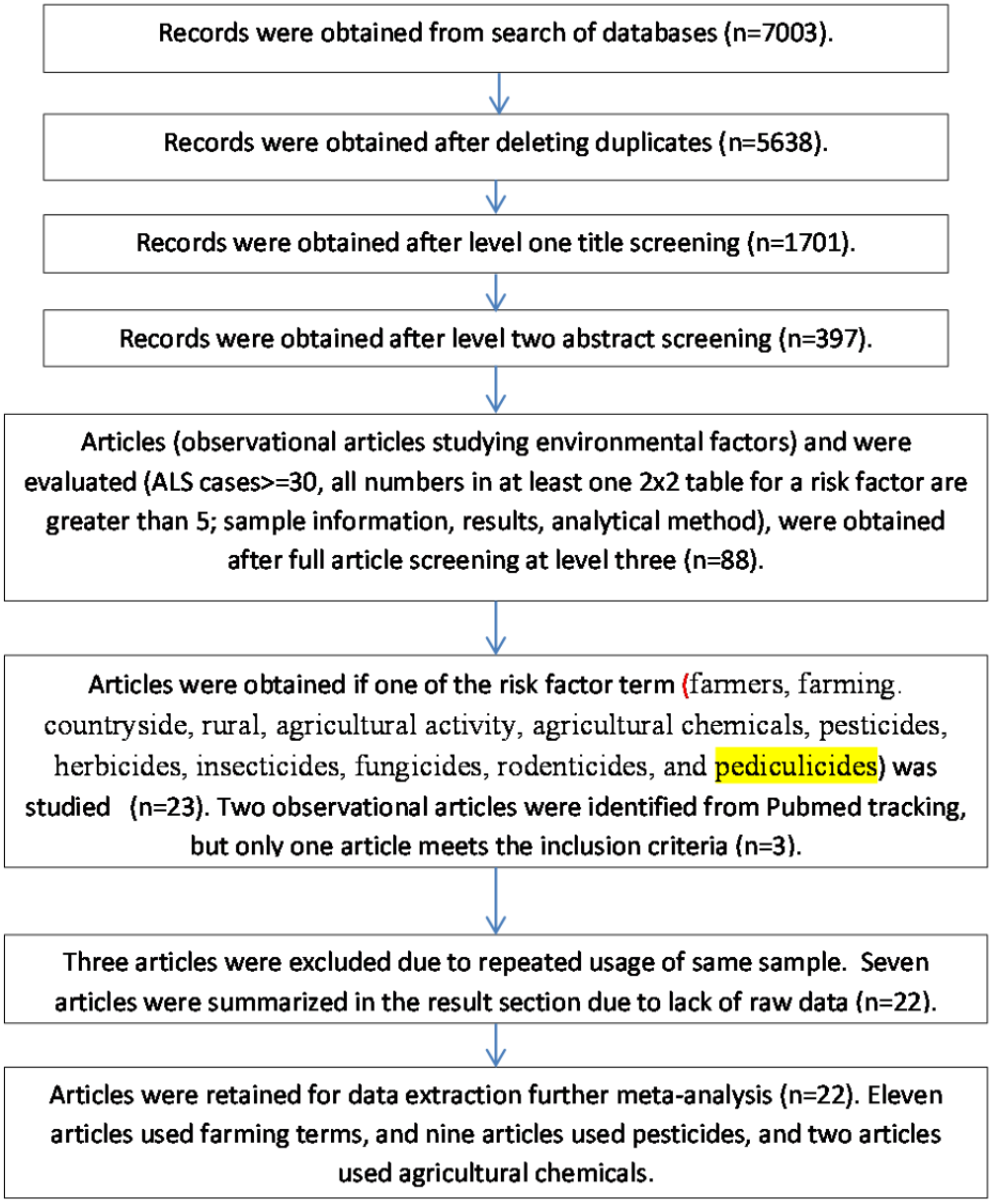

Figure 1: Literature search, screening, evaluation, data extraction, data analysis flow chart.

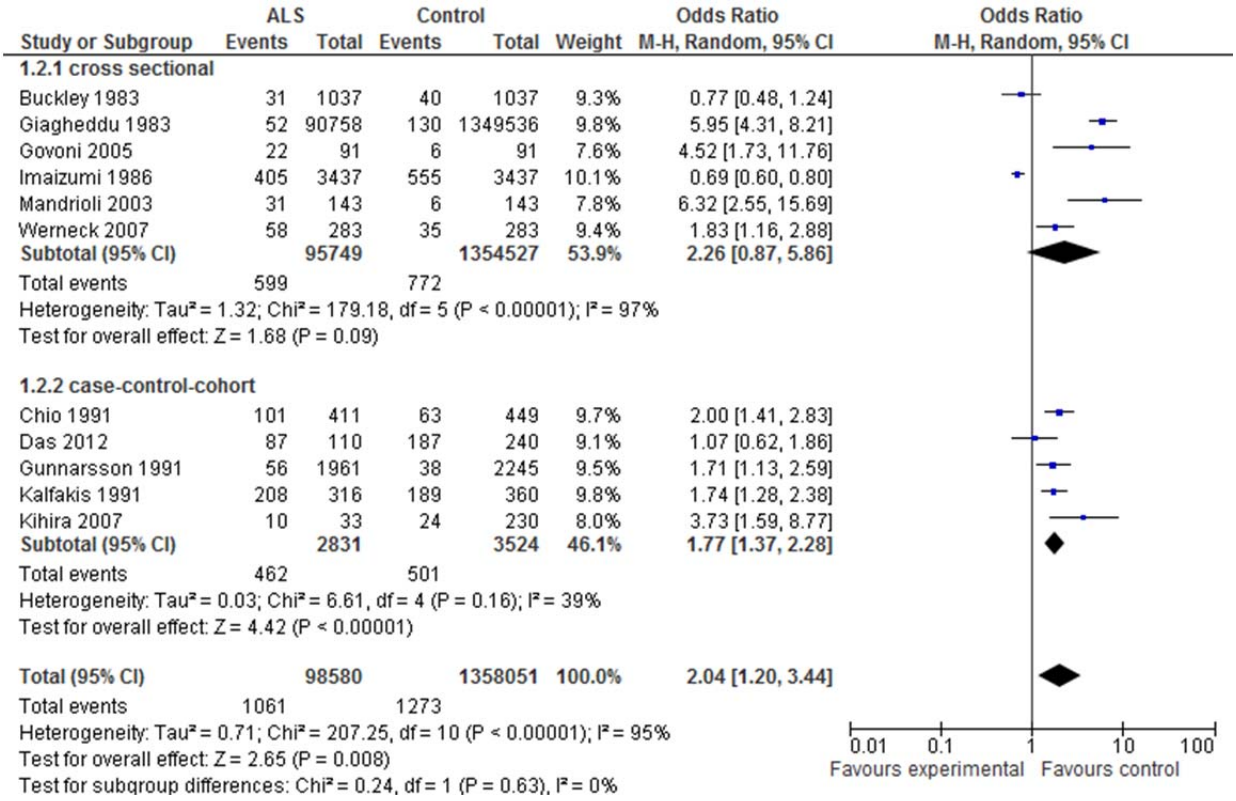

Figure 2: Cross-sectional studies contributed heterogeneity significantly. The relationship between previous agricultural occupation and ALS was estimated separately based on cross-sectional studies (top panel) and case-control studies (bottom panel) using revman 5 with random effects model. The significant publication bias was also noticed (data not shown).

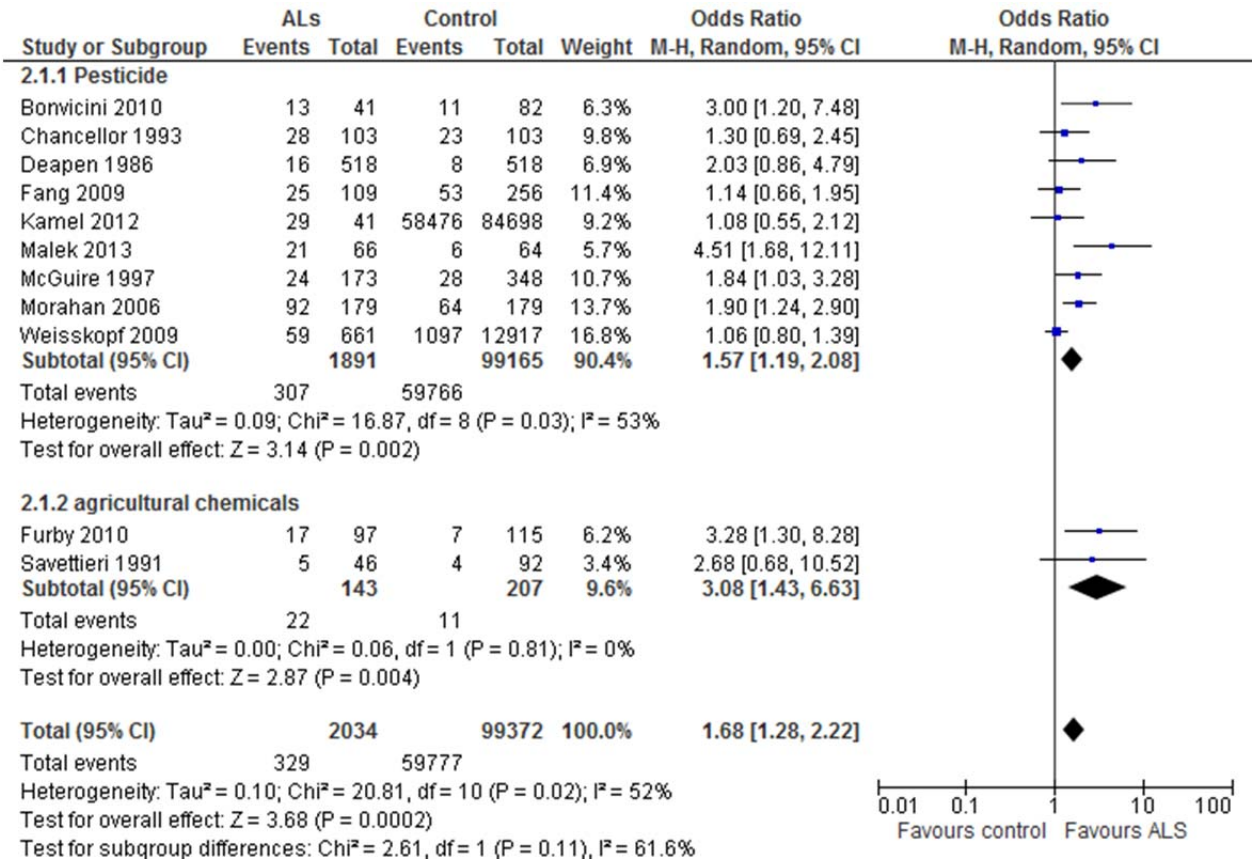

Figure 3: Previous exposure to pesticides/agricultural chemicals is associated with ALS. The relationship between previous exposure to pesticides (9 articles) or agricultural chemicals (two articles) and ALS was estimated using revman 5 with random effects model. Significant publication bias was not noticed (data not shown).

Table 1: Summary of included studies related to ALS onset and agricultural activities

Author, Year, country	Study type	Participants	Characteristics	Pesticide
Cross-sectional studies for farming				
Buckley J, 1983, England	Cross-sectional	1. Deaths from motor neuron disease in England and Wales from 1975-77, classified by age, sex and district were obtained. 2. The original diagnoses of MND cases were cross-examined with ICD by neurologists, found the accuracy (sensitivity) was at 72%.	Cases=356 + 346 + 335, from mortality data	Farmers (including farmers, forest, fish men, cross-section) did not have increased mortality due to ALS. Observed ALS cases=31, expected is 40 (out of 1037 deaths)
Giagheddu M, 1983, Italy	cross-sectional	Surveillance data	From 1957 to 1980 in Sardinia, 182 cases of ALS	Mean yearly ALS incidence per 100,000 active workers in Sardinia from 1957-1980 with relation to occupational status P.Y cases Incidence(per 100000) Farming : 90,758 52 2.39 Factories: 147,990 23 0.65 Other activities: 184,115 20 0.45 Housewives : 343,642 41 0.50 Other non-active population: 673.789 46 0.68
Govoni VJ, 2005 Italy	cross-sectional	1. From 1964-1998	1. 91 incident ALS cases in the LHD of Ferrara in the years 1964–1998 from medical records were identified. 2. Contacts or relatives of all 91 patients were contacted to confirm the information when they were diagnosed (residence information, occupation,) 3. Total 91 incident cases	Farmer-agricultural activity(cross-section) groups Proportion (%) of study population the expected cases observed cases (95% Poisson CI) Agriculture 6.6% 6.0 22 (13.8–32.3) Industry 12.9% 11.7 12 (6.2–20.9) Trade 8.2% 7.5 11 (5.5–19.7) Tertiary 12.7% 11.6 19 (11.4–29.7)
Imaizumi Y, 1986, Japan	cross-sectional	Surveillance over a 10 year period(1969-1978)	Totally 3437 ALS cases were reported from surveillance data.	The death number in agriculture workers only (obs=405/exp=555) or agricultural related workers (obs=353/exp=453) significantly less than expected,

				whereas selfemployed workers were associated with excess death (obs=487/exp=423). 3473 reported cases.
Mandrioli J, 2003, Italy	cross-sectional	1. Identified the patients from all neurological centers, hospitals, death certificates, Italian ALS association. 2. All related clinic records were reviewed (to check the occupations)	1. 143 cases, 67/76=men/women, bulbar/limb=51/87 2. Strenuous physical activity: 54 cases Residence in mountainous area: 26, 18.18%	Farmers-rural residence (cross-section) 1. At least 21.95% of patients were employed in agricultural industry, compared to only about 2.34-6.26% of working population were involved in agricultural activities in the province of Modena, P<0.0001. 2. 15.45% of ALS patients lived in mountainous areas, whereas rural/mountainous residents in these areas made up 9.7–9.9% of the Modena population, the difference was also statistically significant (p=0.021).
Werneck LC, 2007, Brasil	cross-sectional	1. all the cases (283) diagnosed as motor neuron disorders attended a clinic between 1977 and 2004, 32 were excluded.	Sporadic ALS: spinal onset, 144 males, 76 females; bulbar onset, 9 males, 15 females. Familiar ALS: 4 males, 3 females.	Famer-Agricultural workers(cross-section): 1. Excess ALS risk was observed in agricultural workers (cases=58, SMR=1.89). Observed (%)=23.11, expected(%)=12.23, thus expected 35 cases.
Case-control or cohort for farming				
Chio A, 1991, Italy	Case control-control	1. Cases =512 were identified from 1960-1982 from a hospital record consecutive patients. Familiar cases were excluded. 2. Controls=512 with various types of diseases, but not ALS. Admitted into this hospital, admission time, sex, age within 10 years, and residence, matched.	Demographic factors (sex, age, marital status) were similar in both groups	Farmer (case-control) Occupation farmers and breaders was associated with higher ALS risk (exposed/unexposed), cases=101/411. controls=63/449, OR=1.6(1.3-1.9))
Das K, 2012, India	Case-control	1. 110 cases of definite ALS with 240 age and sex matched controls. from Jan. 2008 to Jan. 2011 in the Burdwan Medical College and Hospital, situated in the eastern part of India. 2. Detailed history was taken from every case and control on the basis of open ended structural interview. 3. Diagnosed with EI Escorial.	1. Cases: 95 males, 15 females; controls: 199 males, 41 females. 2. Age range from 15-80 years, no difference between cases and controls	Farmers: 87 in cases; 187 in controls
Kalfakis N, 1991, Greece	Case control	1. 316 patients, 360 controls were recruited from a hospital at Athen at same period (during 1964-1988)	No data	ALS (farmers/non-farmers)=208/108 Control(farmers/non-farmers)=189/171 OR=1.74(1.43-2.05)
Kihira T, 2007, Japan	Case control	1. Consecutively admitted in to Wkayama Medicla Hosapital from 1999-2004 2. The population in the prefecture is about 1 million, more 20% are 65 or more years old. 3. This Hospital has diagnosed about 50% of ALS patients in this Prefecture.	1. 108 patients (definite and probable), 67 males, 41 females. Average age is 61.5±10.4 for both sexes. 2. 244 controls: 146 males, 156 females. Age is at 62.1±10.8 and 64.0±9.9 for males and females respectively 3. 302 neurological patient controls, 40 or older were recruited.	famers-Agricultural occupation(case-control) Primary industry(agriculture, forestry, fishing): 254 controls, 43 ALS: control(9.50%), ALS at 23.10%, OR=2.69 (1.40-5.16) Cases(yes/no)=10/33, controls=24/230.
Gunnarsson LG, 1991, Sweden	Cohort Study	1. 4 million Sweden residents were born 1896-1940. 1960 census. 1. 1970 -1983 all death cases were identified	1. 1961 ALS cases, 1130 males, 831 females. 1067 males with occupations, 308 females with occupations. 2. 2245 controls were randomly selected. around 250 from every 5 consecutive year birth cohort.1080 males, 1165 females. 1005 males and 429 females have occupations.	Farmers (cohort study) Farmers among ALS: 56, 1.7(1.1-2.7). Famers among controls=38 Also, there was a cluster of male cases in agricultural work in one south-western county (OR = 3.4; 25 cases).

Exposed to pesticides				
Bonvicini, 2010, Italy	Case control	Forty-one ALS patients and eighty-two age- and sex-matched randomly sampled population controls were eventually enrolled in the study.	1. 1995-2006 2. 41 cases, 82 sex, age matched controls	1. More cases (13/41) than controls (11/82) were found to have been occupationally exposed to pesticides for at least six months in their life (31.7% versus 13.4%, respectively), in all cases due to agricultural work activities. 2. The number of exposed subjects was 10 among cases (33.3%) and 8 among controls (13.3%) in males, while in females 3 cases (27.3%) and 3 controls (13.6%) had been exposed. 3. Adjusted RR=4.7 (1.4-15.5); male, RR=5.3 (1.2-23.9), female=3.0(0.4-25.7); Less than 68 y for age RR=2.4 (0.4-15.5), more than RR=6.2(1.2-32.7).
Chancellor AM, 1993, England	Paired Case control	1. Of 147 such patients diagnosed between 1 May 1990 and 31 October 1991, 39 had died before approach was possible. 2. Controls were selected by sex, age, living placed matched	The age and sex of cases and controls were identical, a result of the matching process. There were 61 men (mean age 63, range 35-85 years) and 42 women (mean age 67 range 28-86 years).	Pesticides(case-control): cases/controls 10, cases only 18, control only 13, neither 62 OR=1.4(0.6-3.1) cases(exposed/unexposed)=28/75, controls(exposed/unexposed)-23/80
Deapen DM, 1986, USA	Case control	1. Across USA by mailing to all patients who voluntarily contacting to ALS society of USA or clinicians in private or public clinics to distribute the questionnaires to patients with the disease since August 1977. By March of 1977, 1643 patients returned the questionnaires. The 792 patients still living were mailed to a new questionnaires to collect the information about the risk factor, and provide a list of potential controls who were acquaintances to the patients prior to the diagnosis, and at same gender and age (with 5 years). 2.of the 792 patients, 678 completed the questionnaires. For control, of the 678 contacted, 518 returned the questionnaires.	1. 518 cases, 518 controls 2. 65% are males, 98% are white, 53.3 years old at average. 3. Similar to the remaining 1125 patients (61%, 96%, and 56.1 years respectively)	Pesticides(case-control): Exposed to pesticides 16 in P, 8 in C, OR=2.0(0.5-5.4)
Fang F, 2009, USA	Case control	1. All study participants (cases and controls) were recruited between 1993 and 1996 2. Sequential ALS cases were recruited from two major referral centers in New England. 3. Cases were required to live in New England for at least 50% of the year, to be mentally competent, and to speak English. 71% of eligible cases participated in the study (n = 111). About 85% of the cases were enrolled within 1 year after diagnosis and the remainder within 2 years. 4. Population controls were identified through random telephone screening, no neurological disease, sex, age (30–55, 56–65, and 66–80 years) and region matched. 354 eligible controls were contacted, 270 (76%) were enrolled, 256 completed the entire questionnaire.	1. The median age at diagnosis for cases was 60 years (range, 30–79 years) and the median age at 2 years before interview for controls was 59 years (range, 29–78 years). Diagnosis was made 14.3 months after the onset of symptoms on average. 2. Male: Cases, 66 (60.6); controls, 156 (61.7). Female: Cases, 43 (39.4); controls, 97 (38.3) 3. 27 (25%) of the cases had a bulbar onset and 82 (75%) had a trunk or limb onset.	No pesticides were mentioned, except following chemicals: Over all Smokers Non-smokers Insecticides/Herbicides ctl(253) Cases(109) Ctls(148) cases(77) ctls(105) cases(32) /Fungicides/Fumigants 56 25 28 18 28 7
Malek A, 2013, USA	Case-control	1. SALS patients were recruited from 3 major medical neurology clinics with ALS centers, 2 in Pittsburgh, Pa., and 1 in Philadelphia, Pa., between December 2008 and July 2010. FALS cases, and ALS cases with other	The majority of cases and controls were male (68.2%), Caucasian/White (98.5%) and from W. Pa. (86.4%). The cases (mean ± SD: 57.1 ± 13.2 years) were slightly older than the controls (56.4 ± 13.5 years), with a trend	1. Those with occupational exposure to pesticides potentially had 3.17 times the risk of developing ALS compared with those without exposure (OR = 3.17; 95% CI:1.27, 7.93).

		neurological conditions were excluded. 2. Diagnosed with EI Escorial. 3. Cases and controls were required to speak English. Controls were selected from the corresponding geographic region (W. Pa. or Greater Philadelphia area) and 1: 1 matched to cases by age of onset/first ALS symptoms (±5 years), sex and race. 4. Of the 106 patients contacted for this study, 78 participated, with a response rate of 73.6%. But only 66 cases were used for analysis due to lack of the corresponding controls. 5. Occupations held for at least 2 years since the age of 19 years.	for older cases (p = 0.07).	Paired case-control: ALS, 21 exposed, then 45 unexposed. Controls: 8 exposed, 58 unexposed. 2. After controlling for smoking (ever vs. never) and education (high school or less vs. more than high school), cases reported significantly greater occupational exposure pesticides (OR = 6.50; 95% CI: 1.78, 23.77) than controls.
McGuire, 1997, USA	Case control	1.4-year period 1990-1994 2. ALS cases aged l8 or elder years who were newly diagnosed with ALS in western Washington State were identified through a surveillance system, but the cases who lacked a telephone or did not speak English were excluded. 180 cases were eligible, 174 cases agreed to participate. 3.Two controls matched to each case according to sex and age (±5 years) were identified from the study counties using random telephone dialling, or for controls over 65 years of age, Medicare eligibility lists for the target counties was used. Overall response rate is 75%.	1. Cases=174, controls=348 2. Men: cases=95, controls=190, account for 54.6% in both group 3. White: case=164, control=329, account for 94.3, 94.5% respectively 4. Age range not difference, but did not provided average age for both groups 5. Marital status was not different 6. Education level might be different significantly (low/high): cases=83/91 cases, controls=124/224 persons.	Pesticide (case-control) 2. Exposed to agricultural chemicals is associated with ALS. 2.1. Both sexes: unexposed/Exposed: cases=149/24, control=320/28, OR=2.0(1.1-3.5) Men: 73/21 169/21, OR=2.4(1.2-4.8) Women: 76/3 151/7 OR=0.9(0.2-3.8) 2.2. Most significant exposed calendar years were during 1950-1980. 2.3. Most sensitive ages exposed to agricultural chemicals were 18-38. 2.4. Dose dependent trend was also observed.
Morahan, 2006, Australia	Case control	1. 179 SALS cases. Did not mention when and how they were selected. Cases have donated NDA samples to Australian MND banks, were recruited by MND association. more than 90% ALS patients national wide have been recruited into this bank. No, response rate. 2. Control: Age ethnicity, and sex matched normal subjects with non-neurological diseases. 141 unrelated to patients, 38 related patients. Among unrelated control, 96 were spouses, 35 community controls, 10 acquaintances. 3. Self-reported questionnaires	1. Cases: 125 males, 54 females, age, M=60, SD=10 2. Controls: 125 males, 54 females, age, M=61, SD=10	Pesticide-Farming activity (case-control) 1. Herbicide/pesticide: 4.18(1.79-7.94), Logistic regression. 2. Farming activity is not associated, but further analysis showed a statistical significance in females. Univariate analysis 2. Herbicide/pesticides: cases, 92/86; control, 64/115 OR=1.57(1.03-2.41); men, cases,84/40; control, 69/56, 1.70(1.02-2.85); female, cases,32/21; control, 29/25;1.31(0.61-2.86)
Cohort studies for pesticides				
Kamel 2012	Cohort-case-control	The cohort was enrolled in 1993–1997 in Iowa and North Carolina and included 52,394 private pesticide applicators (mostly farmers) and 32,345 of their spouses. The questionnaires collected information on lifetime pesticide use as well as demographics, lifestyle, and medical history. Personally applying pesticides was reported by both applicators (99%) and spouses (56%). Mortality data were available for the cohort through February 7, 2010, from state mortality files and the National Death Index.	1. 41 individuals with ALS on their death certificates, 37 as the underlying and 4 as a contributing cause of death. 2. 41 ALS cases to the remaining 84,698 AHS cohort members without ALS (controls), 3. Most applicators were men (97%), most spouses were women (99%), and most of the cohort was white and not Hispanic (99%). 4. Cases: 18 women, 23 men. Controls: 33466 women,	Exposure to pesticides: 1. Cases: 7 cases, no; 33 cases, yes; 1 missing. Controls: 14296 controls, 68772, yes; 1630, missing OR=1.1 (0.4-3.0) (ever exposed/no exposed). 2. Exposure for 25 days or less versus 26 or more for life time: Cases: 18/23; control, 33289/51409. OR=0.8(0.4-1.8)

		ALS cases were reviewed and confirmed by neurologists against ALS diagnostic criteria.	51232 men. 5. No difference between cases and controls was identified for age, education, location, smoking.	
Weisskopf, J 2009, USA	Cohort Study	1. Followed 414 493 male and 572 736 female CPS-II cohort participants who were alive as of 1 January 1989 (earlier ALS deaths were not coded separately), reported no major illness at baseline (1982) and were no missing data on age or sex. 2. ALS deaths were defined as an underlying or contributing cause of death on death certificates of ICD-9 (1989–1998) code 335.2 or ICD–10 (1999–2004) code G12.2 (motor neuron disease) 3. Participants contributed follow-up time from 1 January 1989 to the date of death, or 31 December 2004 (the most recent linkage with NDI), whichever came first.	1The numbers of cases among the exposed in these analyses were: pesticides/herbicides, 18; asbestos, 10; chemicals/acids/solvents, 36; coal or stone dusts, 8; coal tar/pitch/asphalt, 1; diesel engine exhaust, 9; dyes, 13; formaldehyde, 22; gasoline exhaust, 30; textile fibres/dusts, 22; wood dust, 8; x rays/radioactive material, 14.	Pesticides/herbicides(cohort) cases person.y adjusted RR(full cohort) RR(restricted cohort) No 1097 12,917 Ref Ref Yes 59 661 1.07 (0.79 to 1.43) p=0.67 1.44 (0.89 to 2.31) p=0.14
Agricultural chemicals				
Furby, J, 2010, France	Case control	1. This is a rural area with a rather stable population of about three million inhabitants. All subjects had been living in Brittany for more than 1 year. 2. 2006–2008. 3. The 108 patients, 122 controls were enrolled consecutively from the orthopedic service of Saint-Brieuc Hospital where they had been hospitalized for minor traumas. Controls were age and sex-matched to the patients. Within the control group, all subjects with chronic neurological disease or alcohol consumption were excluded.	Age at interview: Patients , M=68 SD=18.0 range[34–86] ; Controls, M=65; SD=18.0 range[31–84] . Sex men/women: Patients , 59/49 ; Controls 68/54.	pesticides-Agricultural chemicals(cases-control) Agricultural chemicals (yes/no): cases= 17/91, controls=7/115 3.043 [1.194–7.756] p=0.020
Savettieri G, 1991, Italy	Case control	Not described for recruitment, no response rate mentioned study period was not included. Controlled were age, sex, social economic status, living place matched	No further information 46 cases 92 age, sex, residence place, and social economic status matched	pesticides-Agricultural chemicals (case control) (exposed/non-exposed): cases=5/41, controls=4/88, OR=3.00(0.4-20.3)
Excluded studies from meta-analysis				
Cruz DCN, 1999, USA	Case control	1. From 1990-1994, 180 incident ALS patients were identified in 4 Western Washington state (4 counties). 174 agreed to participate this study. 20 case patients died before the interviewed, but the information was provided by surrogates. 2. Two controls were selected for each by matching gender and sex within 5 years. First cohort of controls was selected by random telephone dialling, 221 out of 267 eligible controls agreed to participated. Second cohort was selected from medical care eligibility. 121 out 202 controls agreed to participate.	1. Males/females=95/79 2. White/non=163/11 3. Age range 18->75; close to controls, marital and educations were also close	Rural residence (case-control): not associated. Ever Rural residence(yes/no): cases=72/102, controls=151/197, OR=0.8(0.6-1.3)
kondo 1981, Japan	Case control	1. Cases were identified from multiple sources, including death certificates. 2. Spouses were served as controls	1. Cases: 458 men, 254 women. Controls, their wives, or husbands. 1965-1966	Rural residence (case-control) 1. Nonurban residence from birth to 20. RR=0.98 for males, RR=1.33 for females

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<td>3. Spouses who have lived with patients since their marriages provided information. If patient who had no spouse to provide this information, or unmarried patients were excluded
4. Spouses were interviewed by a visiting nurse for the events from marriage to the disease onset (not diagnosis) Study B, case-control, age, sex, and residence matched, but the patients and controls were interviewed by neurologists in study B.</td>
<td>2. Study B:1973</td>
<td>2. Nonurban residence from 20 to onset. RR=1.01 for males, RR=1.44 for females</td>
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<tr>
<td>Qureshi MM, 2006,USA</td>
<td>Case control</td>
<td>1. Between April 1998 and August 2002, recruited 95 subjects with ALS and 106 healthy control subjects in this study.
2. Cases were ide3ntified from clinic.
3. Controls were non-blood relatives (spouse), friends, unrelated subjects (age matched).At recruitment controls were matched to the ALS subjects by gender and age.</td>
<td>Cases Controls
Gender – Males 60 (63.2 %) 58 (54.7%)
Race – Caucasian 91 (95.8 %) 102 (96.2 %)
Mean age at enrolments 54.4 ±13.1 (SD) 52.5 ±14.9 (SD)
Weight (Kg) – Females 66±19 (SD) 65 ±10 (SD)
Weight (Kg) – Males 82 ± 13 (SD) 86 ± 17(SD)</td>
<td>1. Toxin including pesticide (case-control, but not detail exposure data in cases and controls). 1. Toxin exposure** 2.82 (1.5–5.18) toxin included lead, pesticides, solvents.
2. After adjusting for smoking, welding, gender, age and military history, we found that approximately 45% of the ALS subjects, but only 23% of controls, had been exposed to a toxin.
3. Of the 95 subjects in the ALS group, the toxin exposure most commonly reported was exposure to pesticides (n=18), followed by lead (n=15), industrial solvents (n=5), mercury (n=5) and miscellaneous toxins.</td>
</tr>
<tr>
<td>Park RM, 2005, USA</td>
<td>Cohort Study</td>
<td>1. Death certificate information for all deaths from 22 participating states in the years 1992–1998 was obtained using the National Occupational Mortality Surveillance System
2. Count Underlying and contributing causes of death
3. Controls were all decedents with no mention of neurologic disease, degenerative or otherwise, and excluding: accidental causes, malignant neoplasms of the brain (ICD 191), other senile and presenile organic psychotic conditions (ICD 290), diseases of the nervous system and sense organs (ICD 320–389), and finally, neoplasms of the lymphatic and hematopoietic tissues (ICD 200–208), due to suspect associations with solvents or electromagnetic fields (EMFs).</td>
<td>
<table>
<tr><td></td><td>Motor neuron disease related death</td><td>Total deaths</td></tr>
<tr><td>White men</td><td>3,851</td><td>1,479,921</td></tr>
<tr><td>White women</td><td>2,152</td><td>803,110</td></tr>
<tr><td>Non-white men</td><td>203</td><td>203,862</td></tr>
<tr><td>Non-white women</td><td>141</td><td>127,453</td></tr>
<tr><td>Total</td><td>6,347</td><td>2,614,346</td></tr>
</table>
</td>
<td>Pesticides(cohort)
Total death ALS MOR
3. All farm-related: 149,562 245 1.20(1.02-1.41)
4. Pesticides: 147,688 240 1.20(1.02-1.41)
5. Farmers, excl horticultural 120,193 198 1.23(1.03-1.46)</td>
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Chapter 6: Previous traumatic injury could be a risk factor for ALS –a systematic review and meta-analyses of observational studies

Ming-Dong Wang[1], Julian Little[1], James Gomes[1], Neil Cashman[2] and Daniel Krewski[1]

1. Department of Epidemiology and Community Medicine, Faculty of Medicine, University of Ottawa, Ontario, Canada, K1H 8M5

2. Department of Medicine, University of British Columbia, Vancouver, BC, Canada V5Z 1M9

Abstract: Although the association between trauma and amyotrophic lateral sclerosis (ALS) has been suspected since the first cases of ALS were reported, a causal relationship between previous trauma and the risk of ALS has not been well established. In this study, we systematically reviewed and synthesized data from observational studies on the association between ALS and trauma, as well as with other related risk factors, such as organized sports, strenuous occupation, lower education and lower body mass index, and reported our findings according to the PRISMA guideline. A meta-analysis of 15 included studies revealed a significantly increased risk of ALS [OR=1.64(1.36-1.98)] in relation to reported previous trauma. ALS was also found to be associated with a reported history of bone fracture [OR=1.24(1.05-1.47)] and head trauma [OR=1.27(1.02-1.57)]. This increase in risk remained apparent in analyses restricted to individuals with traumatic injuries reported to have occurred at least 5 years prior to ALS diagnosis [OR=1.40(1.06-1.86)], a finding mitigating against a reverse causality hypothesis. The maximum attributable risk among ALS patients due to previous trauma was estimated to be 4% of sporadic ALS cases. The relative risk for ALS was increased among individuals participating in professional sports [OR=1.35(1.11-1.65)], and among those with blue collar occupations [OR=2.86(2.10-3.90)]. These associations may be partially explained by previous trauma, since individuals with these

occupations may not only have a higher probability of being injured, but also tend to have lower BMI and socio-economic status. Consistent with this expectation, meta-analyses showed that ALS cases had a lower body mass index (measured before ALS onset) than controls [Risk difference=-0.24 (-0.34 to -0.14)], and lower educational status [OR=2.04 (1.58-2.06)]. However, participation in general sporting activity or physical exercise was inversely associated with ALS [OR=0.90(0.78-1.03)].

Introduction

Amyotrophic lateral sclerosis (ALS) is a rare progressive neurodegenerative condition of motor neurons. It selectively paralyses skeletal muscles in affected individuals (28). The aetiology of ALS is largely unknown, except for a small proportion (about 10%) of ALS cases that are caused by monogenic mutations in about two dozen genes (14). In addition, polygenic variation, including polymorphic gene variants, also is thought to play an important role in the development of ALS (65,111-114). Heritability studies suggest that environmental risk factors and genetic risk factors play about equally important roles in the development of ALS (17,18). However, no single environmental risk factor has been conclusively associated with ALS, although heavy metals, pesticides, electric shock, and mechanical trauma have been reported to be associated with ALS in a number of studies (12,120,154).

Previous trauma that could directly or indirectly affect the nervous system has been reported to be linked to the development of ALS since the first ALS cases were reported (406). Recent small scale pathological studies suggested that there was an association between repetitive chronic brain injuries and ALS (173,174,407). In particular, in one of these, evidence of chronic traumatic encephalopathy (CTE) was found in 68 out of 85 subjects with histories of repetitive mild traumatic brain injury and eight subjects (12%) with CTE had been diagnosed with ALS, a significantly higher proportion than in controls (13). Overall, most of the studies completed to date suggest that repetitive traumatic injury is a risk factor for ALS, including studies in Italian soccer players (180), American football players (184,408), and war veterans (207). Additional support is derived from case reports (409,410), with cases of ALS following head trauma having been reported since a century

ago (326,410). Riggs et al have followed reported ALS cases for many years (410,410-412), and consistently concluded that trauma was associated with ALS (412).

Nonetheless, the epidemiological association between trauma and ALS has not been well established, with neither previous systematic reviews nor meta-analyses having been able to resolve this issue (126,135,413). In this article, we report the results of a comprehensive systematic review and meta-analysis of the association between ALS and trauma, as well as other potential risk factors for ALS.

Methods and materials

This systematic review was reported following the PRISMA guideline (25).

1. Search strategy and databases searched: The search strategy was developed in Medline using search terms which were carefully selected to find relevant scientific publications, including systematic reviews, meta-analyses, and observational studies (case-control, cohort, and cross-sectional). Once the search strategy was finalized in Medline, it was adapted to search other databases. The search terms considered include disease relevant terms (amyotrophic lateral sclerosis, ALS, motor neuron disease, MND, Lou Gehrig's disease), and search terms focusing on potential environmental risk factors for ALS (see supplemental material). The different databases that were searched included: Medline, Pubmed, EMBASE, Toxline/toxnet, Ageline, Proquest, Psycinfo, and Google Scholar. Relevant articles from the reference lists of selected articles were also retrieved and reviewed.

2. Article search and selection criteria: The lists of articles identified by searching the different databases were pooled within Reference Manager. Duplicate articles were removed by comparing references with the authors' names, article title and publication year. The remaining articles were exported into DistillerSR, within which all articles were screened by reading the titles and applying the inclusion/exclusion criteria [English, human study, disease terms (ALS, or MND, or amyotrophic lateral sclerosis, or motor neuron disease, or Lou Gehrig's disease), and observational epidemiological study (cohort, case-control, cross-sectional)]. Generic reviews, commentaries, letters to the editor, clinical trials, and laboratory

experiments with non-human subjects were excluded by the primary reviewer; excluded articles were evaluated by a second reviewer who randomly reviewed 5% of the excluded articles. Following completion of first level screening, relevant articles were screened at second level, which involved reading the abstracts and applying the inclusion criteria as mentioned above. The articles retained after second level screening entered into level 3 screening by reading the full articles against the same inclusion criteria used in the previous screening steps.

Exclusion criteria: Since the aetiology of ALS-PDC (ALS-Parkinsonism-dementia complex, refers to ALS that was first identified in Guam) in Guam and the surrounding Pacific islands, an area with an incidence at least 50 times higher than the global average during 1960s-1970s, while not known, is likely to be mainly associated with a local factor, thus articles that dealt with ALS-PDC only were excluded. In addition, ALS diagnosed in veterans might be associated with specific military related risk factors. Enrolment in military service is highly selective and so the profile of exposures and confounders is likely to be different to the general population. Therefore, articles related to military service only were also excluded.

Inclusion criteria: The remaining articles were included on the basis of sample size (> 30 cases, >30 controls), and provision of sufficient information about diagnostic criteria, information on environmental exposures, data analysis methods, and clarity of description of the results (24). A total of 88 articles on environmental exposures were retained for data extraction using a predesigned form in DistillerSR. These articles were searched for the terms 'injury, trauma, and related factors such sports, strenuous work, education, BMI, and bone fracture'. An "antecedent" injury or trauma is defined in present study as an injury that was reported to have required medical attention and occurred at least two years prior to the diagnosis of ALS in cases, or two years prior to the interview of control subjects. An 'old' trauma or injury was defined as any injury occurring at least 5 years prior to ALS diagnosis, or 5 years prior to interview in control subjects**.** Strenuous work refers the some occupations with repetitive laborious job, such as construction, mining, heavy machine operators.

3. Data synthesis: Since ALS is a rare neuronal degenerative condition of motor neurons, most observational studies are performed with case-control designs, or combined case-control/cross-sectional designs. There were some prospective studies not originally designed to examine risk factors for ALS, as well as some studies based on death certificates and case registry. Meta-analysis was used to quantify the association between ALS and exposure based on the results of the published case-control and cohort studies, using the odds ratio as a summary measure of risk based on a random effects or fixed effects model. Heterogeneity across included studied was examined using the Tau^2 and I^2 statistics. Forest plots of the data were prepared and funnel plots were used to evaluate publication bias in studies selected for inclusion in our meta-analyses.

4. Maximum attributable risk (MAR) due to previous experience of trauma: In order to help the general public to understand how previous experience of trauma associated with ALS, the **MAR** was proposed to determine a realistic upper limit for the attributable fraction of the disease prevalence associated with an exposure to a risk factor in a situation in which there may be multiple causes. This was done using the method given by Breslow and Day's (338). The assumptions underlying the calculation are: 1) disease prevalence in the population is low (usually <5%); 2) previous trauma is causally associated with ALS; 3) ALS cases and controls included in the selected observational studies are representative of the total population of ALS patients and general population; and 4) all excess traumatic events among ALS cases as compared to controls are responsible for the development of ALS among those who reported a history of trauma. Under these assumptions, the MAR is given by

$$MAR\ (\%) = 100 \times p*(r-1)/[p*r + (1-p)],$$

where p denotes as the proportion of persons with a history of trauma in the control population, and r denotes as the relative risk (RR) for previous trauma.

Results

A brief summary of article searching, screening, article evaluation, data extraction and analysis is presented in Figure 1. A total of 36 articles were identified with trauma or injury related articles, including articles using sports, athletics, strenuous jobs, lower education, and BMI as risk factors for ALS (See supplemental table 1). We report the association of ALS with these risk factors separately in following sections.

1. The prevalence of antecedent injuries among ALS patients is significantly higher than in controls. A total 16 case-control studies were identified (93,126,145,167,176,327,330,333,393,414-419). The data from study A of Kondo's article was excluded from further meta-analysis since spouses were selected as controls in this study (393), a different study design from other included studies would lead to a disproportionate ratio of males versus females in pooled population, and significantly contribute to the heterogeneity across studies included in the meta-analysis (I^2=97%, Figure 2.1). Meta-analysis of the remaining 15 case-control studies showed that the proportion of ALS patients reporting a history of injury was significantly higher than the proportion among controls (OR=1.64, 95%CI: 1.36-1.98, random effects model) with no significant heterogeneity across included studies [Tau² = 0.05, Chi² = 24.69, df = 15 (P = 0.04), I² = 43%) (Figure 2.2). Results using a fixed effects model were virtually identical (data not shown).

If the included studies were divided into newer studies (published after 1990) or older studies (published in 1990 or before), heterogeneity was reduced among the newer studies (OR=1.45(1.13-1.86); heterogeneity, p=0.15, I^2=35%) and among the older studies (OR=1.90(1.44-2.50), heterogeneity, p=0.08, I^2=0.46), compared to mixed group.

2. The prevalence of old injuries among ALS patients is significantly higher than that among controls. An 'old' trauma or injury was defined as any injuries occurring at least 5 years prior to ALS diagnosis, or 5 years prior to interview in control subjects**.** Data for old trauma were identified from 4 studies (93,393,416,418). The meta-analysis showed that the risk of experiencing injuries at least 5 years prior to diagnosis is significantly elevated among ALS patients as compared to control subjects [OR=1.40, 95%CI: 1.06-1.86, random effects, Tau² = 0.03, Chi² = 4.50, df = 3 (P = 0.21); I² = 33% (Figure 3)], indicating that reverse causality is unlikely the explanation for the association between antecedent injuries and ALS.

3. Previous bone fracture is associated with ALS onset. In order to reduce recall bias based on the injury inquiry in questionnaire, many studies used bone fracture as an objective surrogate for the previous injury to investigate the association between previous trauma and ALS. Eight case-control studies with bone fracture information were identified (49,128,334,367,415,417,418,420). The meta-analysis showed that the previous bone fractures among ALS patients was significantly higher [OR=1.24, 95%CI: 1.05-1.47, fixed effects] than among control subjects, despite significant level of heterogeneity across the eight included studies [Chi^2 = 20.14, df = 7 (P = 0.005); I^2 = 65%](data not shown). The significance of association is diminished somewhat under a random effects model [OR=1.30, 95%CI: 0.93-1.81, Tau^2 = 0.13; Chi^2 = 20.14, df = 7 (P = 0.005); I^2 = 65%](Figure 4) This may not be simply due to the apparent heterogeneity across included studies: even the outlier article was removed, statistical significance was not greatly improved (p>0.05 in random effects model).

4. Previous head trauma is associated with ALS. Nine articles were identified with head injury information. One cohort study showed that the head injury 5 years or earlier prior to ALS diagnosis occurred more frequently (RR=1.23) among a sample of 55 ALS cases (240). Since the study design is different from included case-control studies, thus this study was not included in further Meta-analysis. One case-control study with no raw data was excluded (144). Thus the meta-analysis with remaining seven included studies (126,148,176,207,333,415,418) showed that the prevalence of previous head injuries among ALS patients was higher than in controls [random effects OR=1.27, 95%CI: 1.02-1.57; Tau^2 = 0.01; Chi^2 = 6.57, df = 6 (P = 0.36); I^2 = 9% (Figure 5), a conclusion consistent with an earlier meta-analysis(126). Similar results were obtained with fixed effects model (data not shown).

5. Participating in an organised high school or college athletic club, or in professional sports, but not participating in generic sports or active physical activity, is associated with ALS. Seven articles were identified. One article was conducted and supported by a sport association, show a clear interest conflict, therefore was excluded in the further meta-analysis (178). Six case-control articles were selected to include in this meta-analysis of the association between physical activity or participating in sports and ALS

(49,136,176,179,190,418). The results showed that participating in sports or other physical activities were not associated with increased ALS risk [random effects OR=0.90, 95%CI: 0.78-1.03; $Tau^2 = 0.01$; $Chi^2 = 6.55$, df = 5 (P = 0.26); $I^2 = 24\%$] (Figure 6). Similar results were also obtained from fixed effects model (data not shown). Since heterogeneity is not significant, we can consider this to be a negative association, indicating that actively participating in sport activities may be associated with a reduction in the risk of developing ALS of about 12%.

In contrast to those participating in physical activity and sports, subjects who have ever participated in organized an athletic club in high school or college, or in professional sports, are more likely to develop ALS (136,176,179,418) [random effects OR=1.35, 95%CI: 1.11-1.65. $Tau^2 = 0.00$; $Chi^2 = 3.11$, df = 3 (P = 0.38); $I^2 = 3\%$] (Figure 7). Similar results obtained with fixed effects (data not shown).

6. Strenuous work is associated with ALS. Strenuous work is a collective term associated with an occupation that often requires repetitive hard physical work. Such occupation – including mining and construction, for example, is associated with a high probability of mechanical injury (421). Three registry based studies were identified. Two studies showed a higher risk of developing ALS among workers with strenuous occupation (151,188). One study did not identify a higher than expected risk (422). We also identified two cohort studies. Both showed higher than expected risk of developing ALS [RR=1.7(0.9-3.0) and 1.94(1.30-2.78), respectively]. Since the registration studies and cohort studies have different study design compared to case-control studies, therefore, we excluded them from further meta-analysis. However, the overall results from these five studies show an association between ALS and strenuous work occupation. Thus, a meta-analysis of remaining seven case-control articles (143,176,333,334,415,419,420) showed that the association between strenuous work and ALS was statistically significant [random effects OR=2.70, 95%CI: 1.97-3.69 (Figure 8)], with no significant heterogeneity across studies [$Tau^2 = 0.00$; $Chi^2 = 3.47$, df = 6 (P = 0.75); $I^2 = 0\%$]. Similar results obtained with fixed effects model (data not shown).

7. Meta-analysis of the association between lower education and ALS: Five included observational studies all suggested that lower education was associated with a higher risk of ALS. Of these, one case-control study indicated that for every additional year of education, the risk of ALS could be reduced 15% after controlling confounding factors (OR=0.85, 95%CI: 0.77-0.95). A similar conclusion was reached by Kamel's group in 2002 using same dataset with univariate analyses (186). One cross-sectional study showed an inverse relationship between the incidence of ALS and education attainment (incidence per 100,000 persons =7.2 and 4.8 for lower and higher educated individuals, respectively), with a relative risk (RR) of ALS in the group with lower education relative to the higher education group being RR=1.5 (414). This article was excluded from further meta-analysis, because the raw data could not be identified from the article. Therefore, the final meta-analysis was done with three case-control studies (185-187). The pooled odds ratio from these three studies for lower education versus higher education among ALS and control subjects was 2.04, 95%CI: 1.58-2.62, with no apparent heterogeneity among the three studies (random effects model,$Chi^2 = 0.14$, df = 2 (P = 0.93); $I^2 = 0\%$) (Figure 9). Similar results were obtained with fixed effects model (data not shown).

8. Meta-analysis of the association of ALS with lower BMI: All five selected studies showed that ALS patients were slimmer when compared in relation to their body mass index (BMI) prior to disease onset (49,176,196,198,219). Among these studies, one study provided categorical data (196), but showed a strong adjusted association [OR=2.10 (1.08–4.07)]. Another article may be a duplicate use of same sample (219). We therefore pooled the data from the remaining 3 studies. The standard mean difference (SMD) in BMI between ALS patients and controls is negative in all three included studies (random effects model, SMD=-0.24, 95%CI: -0.34 to -0.14) with no heterogeneity across three studies (Heterogeneity: $Chi^2 = 0.61$, df = 2 (P = 0.74); $I^2 = 0\%$) (Figure 10), indicating that the ALS patients were slimmer before disease onset. This conclusion is further supported by a large pooled study of data from seven existing cohorts (all participants entered into the cohorts as normal subjects with BMI estimate, then mortality was followed), in which the possibility of reverse causation was essentially ruled out since even if the cases from first 7 years of follow-up period after entry into the cohorts were excluded, the negative association remained statistically significant (197).

9. Maximum attributable risk (MAR) due to previous trauma: A total of 795 ALS cases from a population of 2,243 individuals with ALS had a history of trauma, whereas a total of 972 controls from 3,101 normal subjects had a history of trauma based on the included studies that formed the basis for the present meta-analysis. To estimate the MAR due to previous trauma, we first calculate p = 792/3,301 = 0.3133 and r = [(795/2243)/(972/3101)] = 1.1307. Then the MAR is estimated as [0.3133*0.1307/ (0.3133*1.1307+0.6867)] x 100% = 3.9%. With this attributable risk level, we estimate that up to 88 ALS cases among the total 2,243 ALS cases from all included studies might have been caused by previous trauma.

Discussion

The present multiple meta-analyses revealed that the occurrence of ALS was strongly associated with a history of previous traumas, a conclusion independently drawn by a recent general literature review in this area (423). An association between head trauma and ALS was also demonstrated, in agreement with a recent meta-analysis (126). However, trauma is not a strong risk factor for ALS, we estimated that trauma or injury might be responsible for 4% of all ALS cases at most.

Although many epidemiological studies support the hypothesis that previous trauma is a risk factor for ALS, two recent studies provided evidence of reverse causality between ALS and trauma (175,424). Both studies found that the injuries of head or other body parts were not a strong risk factor for ALS, and that the incidence of trauma increased during the peripheral period of ALS disease diagnosis. A strong association between ALS risk and severe head injury less than one year before diagnosis was observed, but not with severe head injury occurred three years or earlier prior to ALS diagnosis (including subtype of head injuries, or repeated injuries) (424). However, a recent case-control study did not supported a hypothesis of reverse causality (418): when the analysis was limited to traumatic events that occurred 5+ and 10+ years before ALS onset, the association between trauma and ALS remained significant. Our meta-analysis also demonstrated a significant association between ALS and old trauma that occurred five years before ALS diagnosis. This also does not support a hypothesis of reverse causality. ALS is a rapid progressive disease; the first clinical

symptoms usually present abruptly, without noticeable warning disease signal. Before the occurrence of first symptoms, ALS patients are like normal subjects. Therefore, injuries that occurred five years prior to the ALS onset are unlikely to be attributable to preclinical ALS development.

a. Establishing an association between injury and ALS requires a large sample size. Although an association between previous trauma and ALS has been suggested since initial case reports (326), a causal link has not been well established (127,413,425). Although new epidemiological continues to emerge (174,418), the evidence is somewhat inconsistent (175,424). ALS is a multifactorial neurodegenerative condition of motor neurons that locate in the brain cortex, brain stem, and spinal cord. The apoptosis of occurred at one of these locations could cause similar motor neuron diseases. Although ALS is a rare neurological condition, it is more complex than other common multifactorial chronic conditions. For example, although diabetes is a multifactorial condition, there is only one type of target cells affected---insulin producing islet cells.

Even if trauma − especially head trauma − is a true risk factor for ALS, excess trauma may explain at most 4% of sporadic ALS, with the actual value possibly being as low as 0.4%; identification of a risk this small would require a sample size between 2179 and 217941 (at 5% significance and 80% power). With the exception of a recently published retrospective cohort study (424), existing studies may have not had sufficient ALS cases permit definitive conclusions.

b. Bone fracture may not a better surrogate for previous injury: Compared to trauma, bone fracture is a more easily diagnosed outcome, with less recall bias. However, considering only fracture would miss most traumas that are related to brain and spinal injuries. Our meta-analysis showed there is no significant association between history of bone fracture and ALS when using a random effects model (OR=1.30(0.93-1.81)), but with a high degree of heterogeneity across included studies (Tau^2=0.13, I^2=0.65). This heterogeneity was mainly attributable to older articles: if all studies conducted before the year 2000 are removed, the synthetic OR increases with the random effects model (OR=1.52(1.21-1.91)), and heterogeneity is eliminated (I^2=0). These analyses indicate that

even an objective predictor variable like bone fracture does not guarantee the consistency across studies. In addition, the time since fracture in relation to the diagnosis of ALS may be critical. For example, fractures occurring 5 years prior to ALS diagnosis were associated with an increased risk of ALS (326).

c. Lower BMI is associated with ALS. At least three studies reported an association between fitness and ALS risk. The first of these publications showed that an inverse association between BMI and the incidence of ALS among college varsity athletes (196). Two follow-up studies confirmed this type of association, showing that the incidence of ALS was less common in CAD patients than in non-CAD patients, and by showing that the BMI was lower, and physical strength was higher, in ALS patients before disease onset (197,240). However, all three studies could not rule out a possible confounding effect from head or spinal injuries. Our analysis also showed that lower BMI is associated with higher ALS risk (Figure 8). Generally speaking, the individuals with lower BMI could be found in army and sports participants who are predisposed to injury, relative to the general population. Thus, lower BMI itself might not represent the real risk factor for ALS.

d. Previous participation in professional sports is positively associated with ALS, but participating physical activity or generic sports tend to be negatively associated with ALS. Participating in sports or physical activities has been associated with ALS in some, but not all early studies (127,135). This association might be confounded by the misclassification of sports in this kind of studies. Here, we included all identified studies related to sports (excluding professional sports), and found that sport activity tended to play a protective role in the development of ALS (Figure 6). This finding is consistent with a recent study from Veldink's group (136), whereas participating in organized or professional sports was associated with ALS occurrence (Figure 7). The question here is why participating in professional sports, but not other physical activity including generic sports, is associated with ALS. The first possible explanation is confounding by exposure to pesticides (139,140,154), which we have discussed previously (Wang et al, unpublished). The second possible reason is that professional athletes have a higher risk of being injured, especially head injury (135). One cross-sectional study showed that professional soccer players, but not professional basketball or bicycle competitors, have a higher risk of developing ALS,

indicating that physical activity/strenuous repetitive work alone, even professional sports (180), may not be a risk factor for ALS. These observations support the hypothesis that trauma, especially repetitive head injury, is associated with ALS since professional soccer players often head the soccer ball. Our meta-analysis showed that participating in physical activity or generic sports tends to be negatively associated with ALS, suggesting that physical activity is not a cause of ALS, supporting a speculation from Veldink's group (426) .

Individuals with lower education tend to hold blue collar occupations, which is associated with 3.5 fold more occupational injuries as compared to white collar jobs (427). Therefore, the associations between lower education and ALS reported here may also have been confounded by injury. Clearly, this represents only an association, rather than a causal relationship, since lower education itself would not in and of itself be a causal pathway for ALS. The risk factors related to lower education, such as occupations with a high risk of injury, might be responsible for this association.

Limitations

Although we have comprehensively evaluated all identified articles related to traumas and ALS, the associations reported here cannot be interpreted as causal regardless of how many articles included in each analysis. Many associations, including those with lower BMI, lower education, blue collar occupations, and participating organized sports, observed in the scientific literature to date, may be confounded by injury. Further studies should therefore carefully control for the possible confounding effects of injury.

Conclusion

In this article, we found that previous trauma, especially head trauma, was associated with ALS, and account for as much as 4% of the burden of ALS in the general population. Previous trauma is also associated with other neurological conditions, such dementia. Since 12% of examined CTE subjects were diagnosed with MND (174), a strongest support for an association between trauma and ALS. If we assume that ALS cases with head trauma also have suffer CTE (224/1092, Figure 5), then 2.4% ALS cases would be expected to have been caused by previous trauma, very close to our estimation of 4% of all ALS cases in this study. If previous trauma is a risk factor for ALS, other associations (participating in professional

sports, working in strenuous occupations, lower BMI/, and lower education) with ALS might be confounded by previous trauma.

Figures and legends

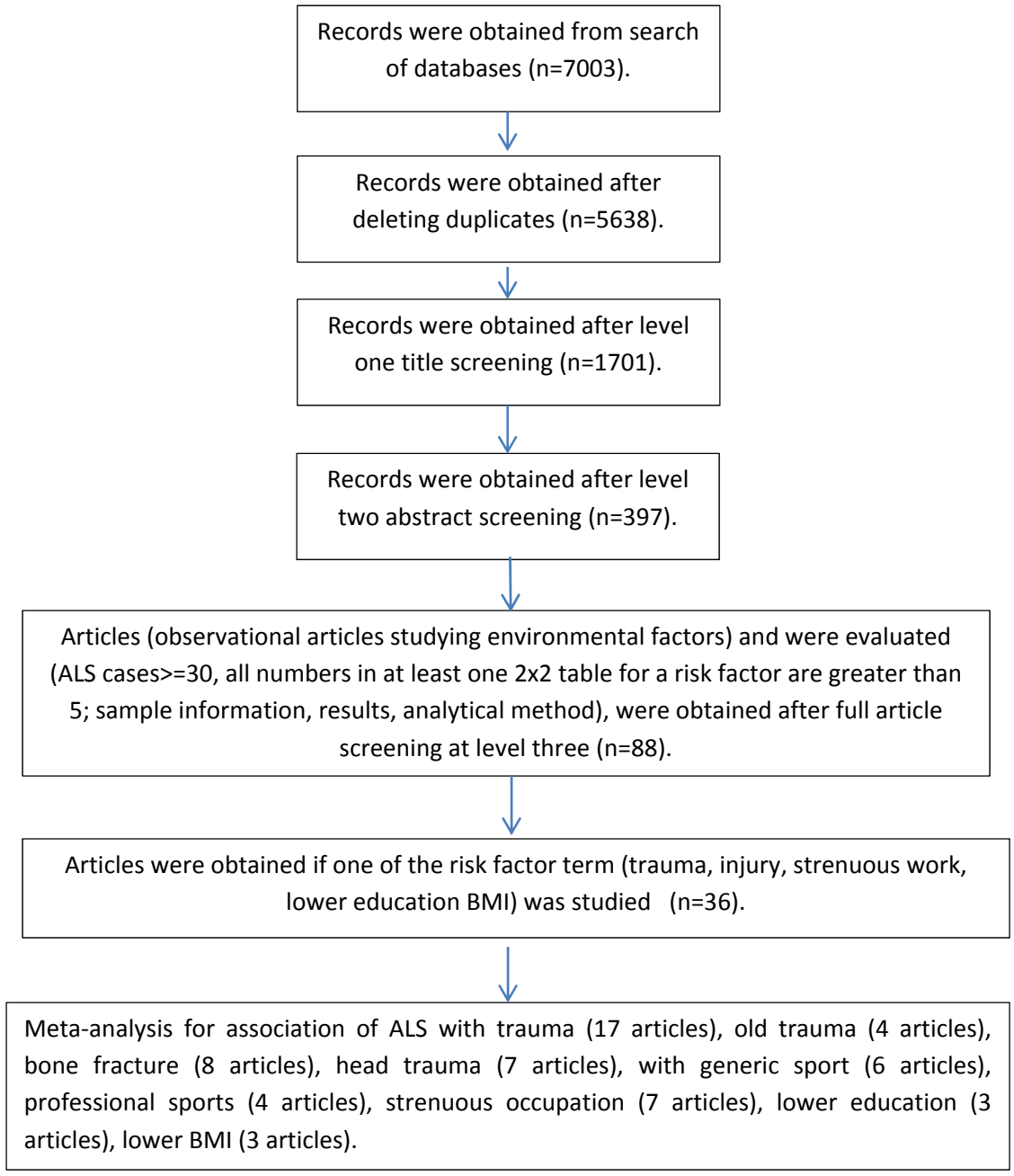


Figure 1: Literature search, screening, evaluation, data extraction, data analysis flow chart (25).

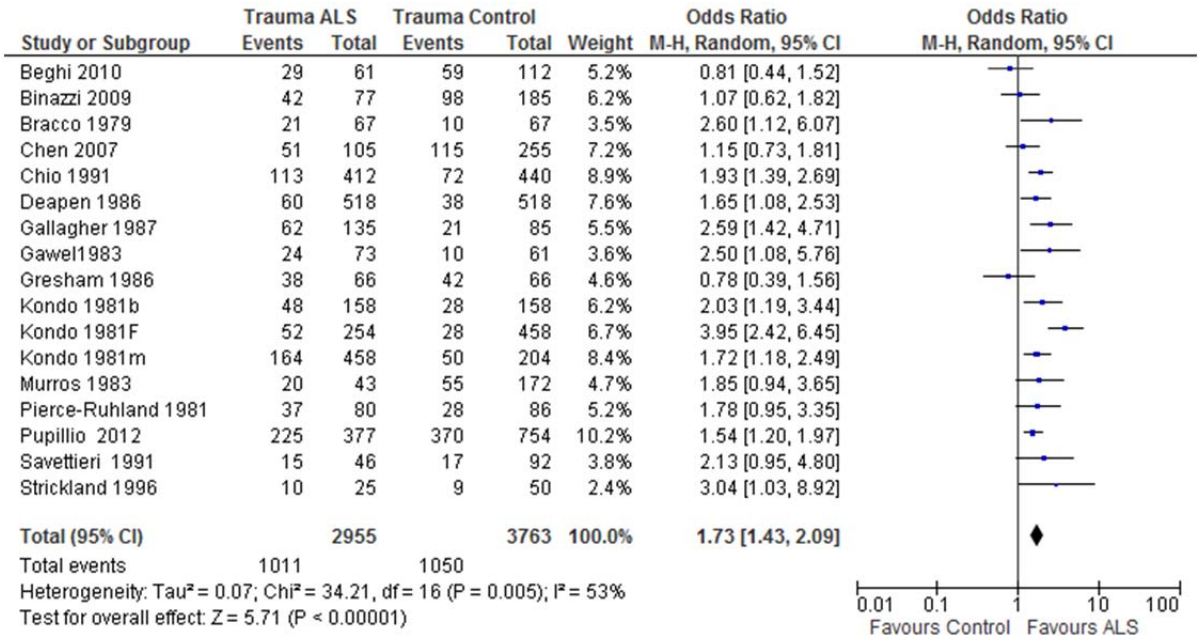


Figure 2.1: Previous trauma is associated with increased ALS risk. Articles related to trauma including any injury causing medical attention and ALS were searched from multiple sources. Data of the injury among ALS and controls from a total 17 studies in 16 articles extracted, the synthesized OR was computed based on the odds of injury among ALS cases and controls with meta-analysis using random effect model of software Revman 5.1. Similar OR estimates were also obtained with fixed effect model (data not shown).

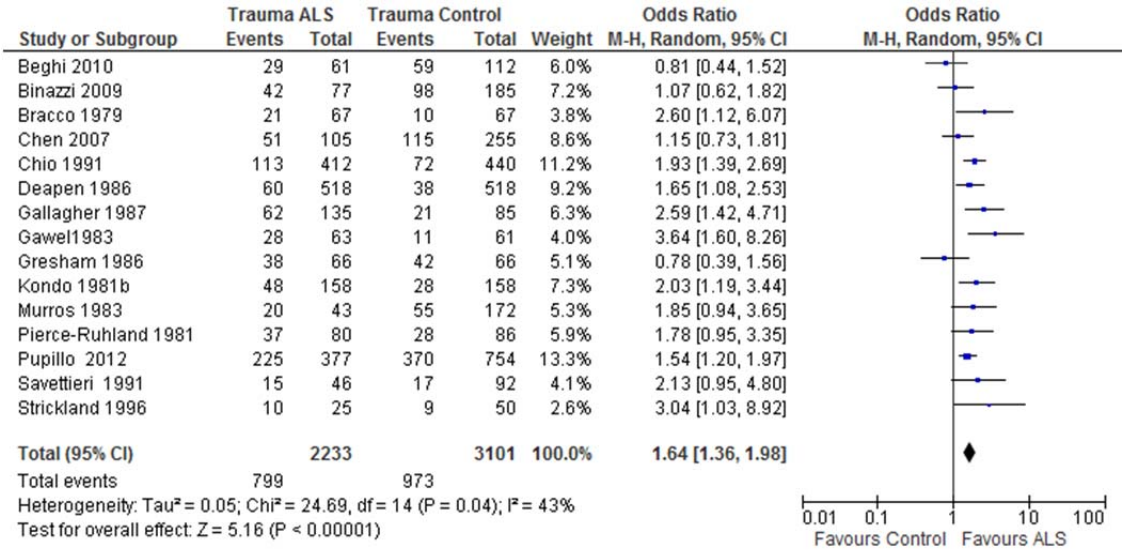


Figure 2.2: Different study design is responsible for higher heterogeneity among included studies. The first Kondo study (1981F and 1981M) was a case-control study with different study design, using spouses as controls. When this article was removed, the heterogeneity across included studies became non-significant, although still high. The synthesized OR was computed based on the odds of injury among ALS cases and controls with meta-analysis using random effect model of software Revman5.1. Similar OR estimates were also obtained with fixed effect model (data not shown).

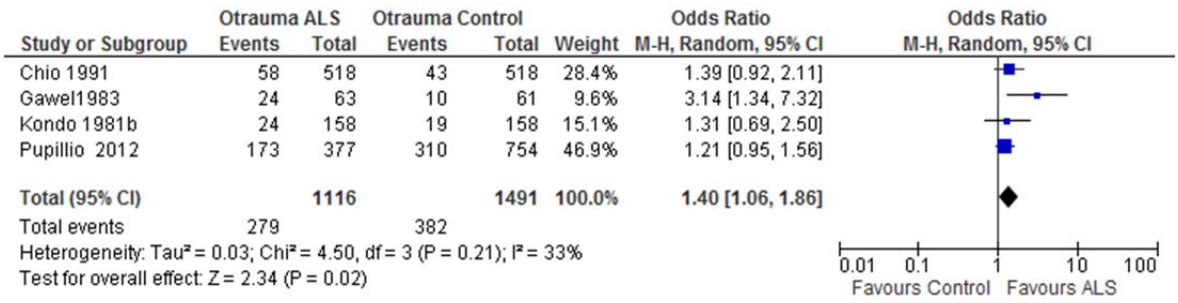


Figure 3: Older trauma is still found to be associated with increased ALS risk. Four articles related to trauma with information of old trauma (injury that caused medical attention) and ALS were identified from multiple sources. Data of the old injury among ALS and controls from these 4 articles were extracted, the synthesized OR was computed based on the odds of trauma among ALS cases and controls with meta-analysis using random effect model of software Revman 5. 1. Similar OR estimates were also obtained with fixed effect model (data not shown).

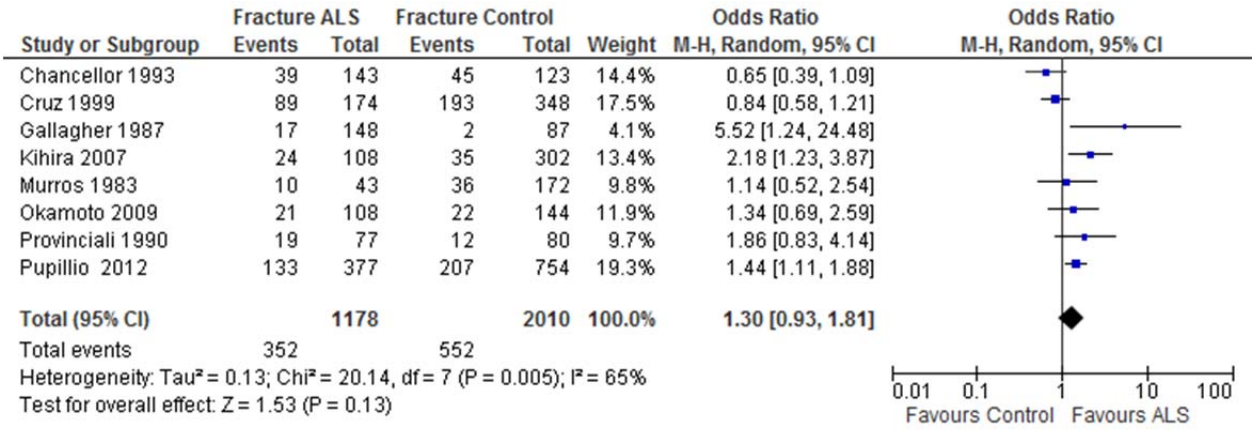


Figure 4: Previous bone fracture is associated with increased risk of ALS. Eight articles related to ALS patients with information of bone fracture were identified from multiple sources. Data of the bone fracture among ALS and controls from these 8 articles were extracted, the synthesized OR was computed based on the odds of fracture among ALS cases and controls with meta-analysis using random effect model of software Revman 5. 1.
Similar OR estimates were also obtained with fixed effect model (data not shown).

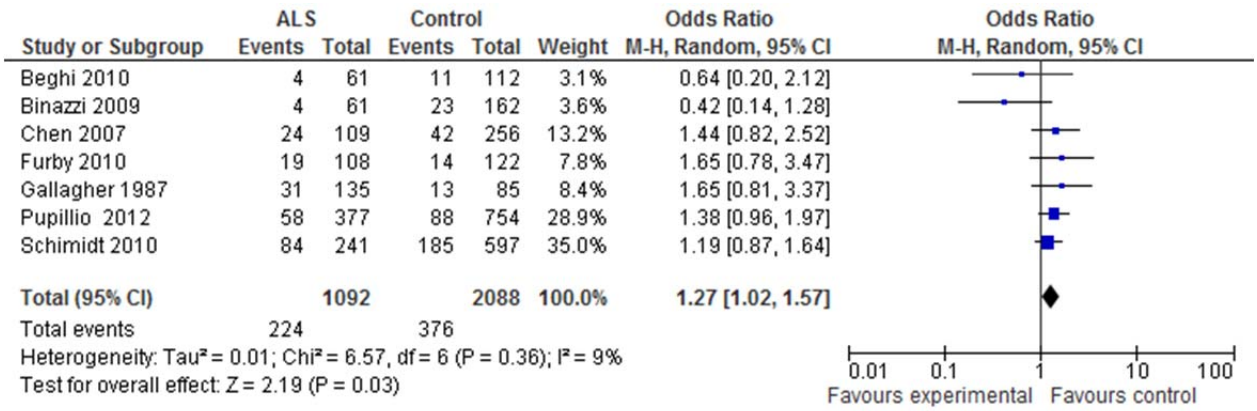


Figure 5. Head trauma is associated with increased ALS. Seven articles related to ALS with information of head trauma (trauma that caused medical attention) were identified from multiple sources. Data of the head trauma among ALS and controls from these 7 articles were extracted, the synthesized OR was computed based on the odds of head trauma among ALS cases and controls with meta-analysis using random effect model of software Revman 5.1. Similar OR estimates were also obtained with fixed effect model (data not shown).

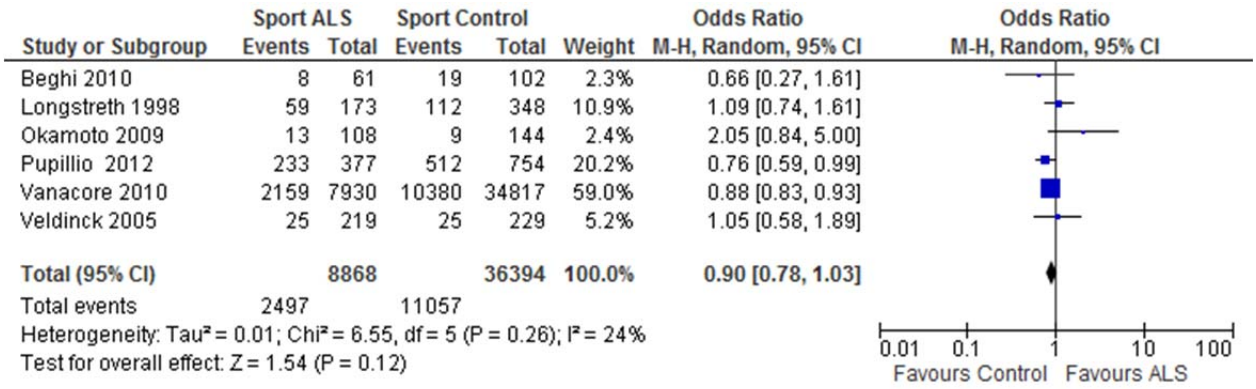


Figure 6: Participating in sports is not associated with increased risk of ALS. Six articles related to ALS with information of sport participation (sport activity 2 years prior to the ALS diagnosis in the cases or 2 years before the study among controls) were identified from multiple sources. Data of participating in sports among ALS and controls from these 6 articles were extracted, the synthesized OR was computed based on the odds of participating in sports among ALS cases and controls with meta-analysis using random effect model of software Revman 5. 1. Similar OR estimates were also obtained with fixed effect model (data not shown).

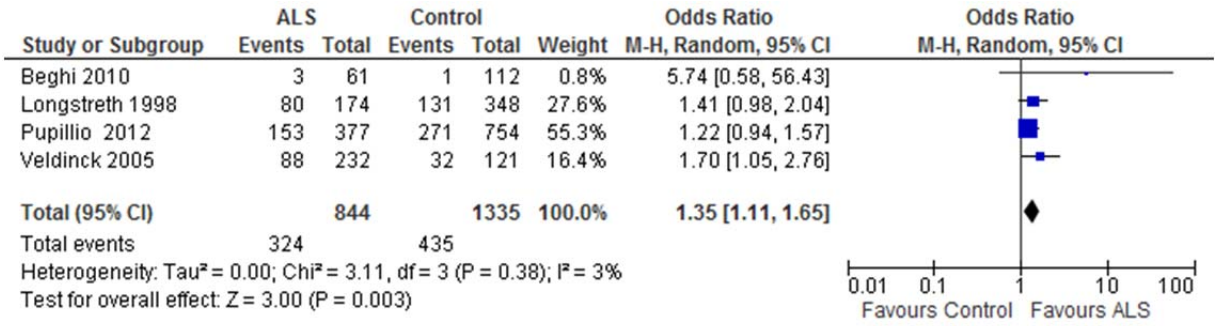


Figure 7: Participating in professional sports is associated with increased ALS. Four articles related to ALS with history of participating in professional sports or organized sports (varsity sports in high school or university) were identified from multiple sources. Data of participating in professional sports among ALS and controls from these 6 articles were extracted, the synthesized OR was computed based on the odds of participating in sports among ALS cases and controls with meta-analysis using random effect model of software Revman 5. 1. Similar OR estimates were also obtained with fixed effect model (data not shown).

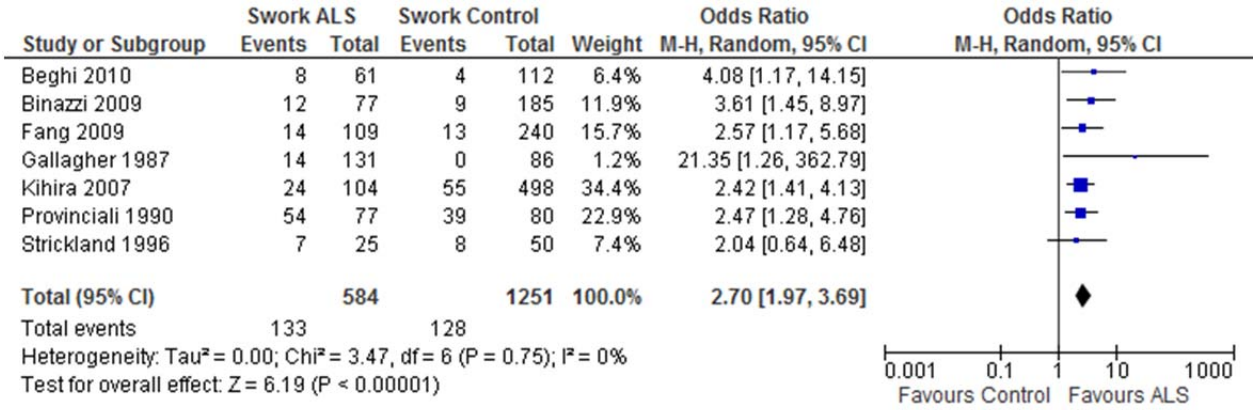


Figure 8: Strenuous work is associated with increased ALS risk. Seven articles related to ALS with information of strenuous work occupation (laborious works related primary and secondary industries) were identified from multiple sources. Data of strenuous work among ALS and controls from these 7 articles were extracted, the synthesized OR was computed based on the odds with strenuous occupation among ALS cases and controls with meta-analysis using random effect model of software Revman 5. 1. Similar OR estimates were also obtained with fixed effect model (data not shown).

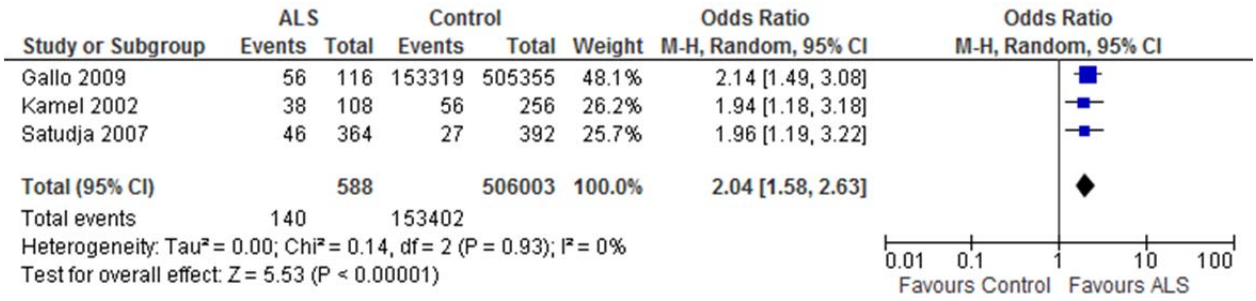


Figure 9: Lower education is associated with ALS. Three articles related to ALS with information of education were identified from multiple sources. Data of lower education among ALS and controls from these 3 articles were extracted, the synthesized OR was computed based on the odds with lower education among ALS cases and controls with meta-analysis using random effect model of software Revman 5. 1. Similar OR estimates were also obtained with fixed effect model (data not shown).

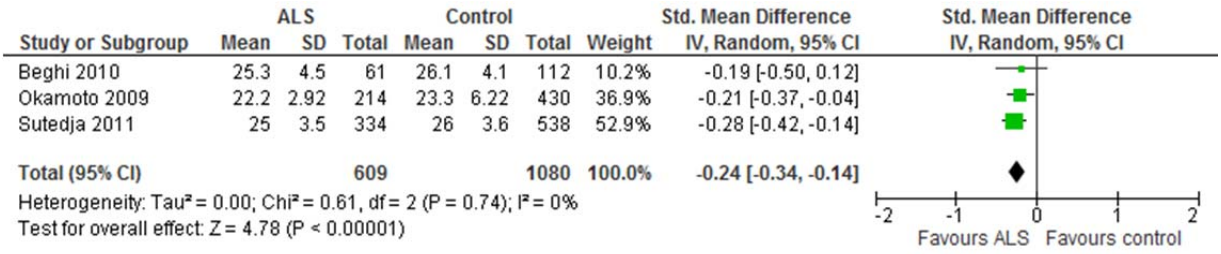


Figure 10. BMI is inversely associated with ALS. Three articles related to ALS with information of body mass index (BMI information 2 years before ALS diagnosis) were identified from multiple sources. Data of BMI among ALS and controls from these 3 articles were extracted, and the synthesized mean difference was computed based on the difference of BMI among ALS cases and controls with meta-analysis using random effect model of software Revman 5.1. Similar mean difference estimates were also obtained with fixed effect model (data not shown).

Supplemental Table 1: Summary of included studies related to ALS onset and agricultural activities

(Note: * indicated that the study was not included in the meta-analysis)

	Author Journal Year	Study type	Participants	Characteristics	Trauma
Trauma or injury	Beghi E, 2010, Italy, UK, and Ireland	Case control	1. P: have to be definite, probable, or possible ALS cases, did mention when the study was done 2. Age (±2.5 y) and sex matched for control for each patient, selected by general practitioners for the patients 3. Report response rate is more than 90%, no further data	1. Cases/controls=61/112. P, 27 F, 34 M; C, 46 F, 66 M, not exactly matched. 2. Age, marital, education, no difference between P and C.	Trauma: head, sport, strenuous workers, professional sports 1. All trauma (yes/no), no difference: P=29/32, C=59/53 2. Head trauma (yes/no): no difference: P, 4/57; C, 11/101 3. Repeated trauma (yes/no): P, 21/40; C, 27/85, OR=1.65(0.97-2.33) 4. Work intensity (Yes/no, P<0.05): Strenuous/all, P=8/53, C=4/108; 5. Sport intensity (yes/no, P>0.05): strenuous/all others, p=8/53, C=19/93 6. Professional sport(yes/no, p=0.04):P=3/58, C=0/112 BMI BMI. cases: 25.3, SD 4.5; controls: 26.1, SD 4.1 Details were not provided in this paper
	Binazzi A, 2009; Italy	Case control	1. Recruited since 17J, 2005 to July 6, 2007. 77 patients, 70 definite, 7 probable. 44 spinal/ 32 bulbar 2. Relatives or accompanying persons of outpatients affected by neurological disease other than ALS, and coming to the same hospital ambulatories, service as population controls 3. No response rate was mentioned	1. Cases, 77; 43, males, 34 females, 44 spinal, 32 bulbar. age=65±9.3, range 42-83. onset ages: 62.4(60.1-64.7), age at diagnosis, 63.7(61.4-66.1). 2. Control: 185 (male=69, females=116), different to cases. Average=57.5±13.0, 28-84.	Trauma-head, leg-strenuous workers 1.Head injury: Years since last injuries: 11-30y, bulbar, OR=3.51 (1.03-11,95), not spinal, not <=10y, or >30y with significance. 2. Ages at last injury: <30y, spinal, OR=7.13(1.34-37.94); 30-40y, bulbar, OR=17.4(1.70-178.5), not other age groups, or onset conditions. 3. Leg injury: OR=1.82(1.01-3.29) for all cases (19/58, control=31/154), other sites, or any site, or separate calculation for bulbar, spinal, no difference. 4. Building or construction: spinal, OR=3.01(1.01-8.99, 6/38; C=6/179); not bulbar, not all cases. 5. Exposed to building and construction materials: spinal, OR=5.27(1.15-24.12, 4/40, C=3/182)
	Bracco L, 1979, Italy	Other	1. Patients: During the 10 years of observation (1/1/1967-31/12/1976), there were a total of 102 cases initially diagnosed as ALS, 83 cases were accepted as ALS and entered into the study. 2. Population-based cross sectional study and case-control study	1.83 patients considered, 46 were males and 37 females; the average at the onset of the disease was 59± 2 years. Considering the sex groups separately, the average age at the onset for the females was 61.6±3 years, while the average age of onset in the male groups was 57 ± 4 years. 2. Did test how the control was selected	Trauma-strenuous work 1. Trauma (presence/absence): cases=21/46, controls=10/57, OR=2.60(2.17-3.06) 2. Heavy labour. The incidence of ALS was increased wish the degree of use of labour forces. p.y cases SMR(standard incidence per 100000) Heavy* 163,045 25 15.3 Medium heavy** 251,250 34 13.5 Light*** 213,693 12 5.6 * Farmers, non- specialized factory workers, maids, athletes. ** Technicians, public transport operators, specialized factory workers, artisans *** Civil service workers, clerks, merchants
	Chen, H; 2007;	Case control	1. Recruitment time and place: 1993-1996 from two major referral centers in New England. 2. Has been diagnosed within 2 years, lived in	1. Sample size: patients: 109/control:255 2. Race and sex ratio might be in their previous papers.	Trauma-head injury-total injury 1. Head injury: OR=1.4 (0.8-2.8), cases: 22.0 per cent, 16.5% in control 2. Total injuries: OR=1.2(0.7-1.9), cases, 46.8%, 45.1% in control

	USA, Boston		New England for at least 50% of the years, spoke English, mentally competent. Control: Random selected via telephone screening, no neurological disease, gender match, age match, at 3:1 for Boston area, and 2:1 for other areas. 3. response rate: patients: 110/154 (two could not be found, 42 declined(7 due to illness, 15 due to travelling difficulties, 20 for other reasons)), 1 missing, final=109 Control: 256 competed questionnaires from 354 contacted, 1 missing, final=255	3. Divided patients into three age groups (30-55, 56-65, 66-80) 4. Study time: 1993-1996 5. Average age at diagnosis, 58.3 y; from self-reported sign to diagnosis is 14.3 months, 27/bulbar, 82 limb onset	3. 1 head injury: OR=0.9(0.4-2.0), cases=11.9%, control=12.5%; >1, OR=3.1(1.2-8.1), p=10.1%, C=3.9%. 4. Years since last injury to ALS diagnosis: <=10, OR=3.2(1.0-10.2), P=7.3%, c=2.4%; 11-30, 1.2(0.5-2.9, p=10.1%, c=8.2%; >30, OR=0.9(0.3-2.7), p=4.6%, c=5.9%. 5. Exclude cases with family history, and the injuries within 3 years from reference date (diagnostic date in cases or 2 years before the interview date), within past 10 years: OR=5.6 (1.3-24.4), no further data/number available.
	Chio A, 1991, Italy	Case control	1. Cases =512 were identified from 1960-1982 from a hospital record consecutive patients. Familiar cases were excluded. 2. Controls=512 with various types of diseases, but not ALS. Admitted into this hospital, admission time, sex, age within 10 years, and residence, matched.	Demographic factors (sex, age, marital status) were similar in both groups	Trauma 1. The number of Trauma in spinal column/upper/lower limbs, and trunk but not head, occurred is higher in patients. 1.1. Total mechanical injuries: cases=113, control=72, OR=1.6(1.3-1.9). 1.2. Frequency of injuries is higher in ALS patients, before diagnosis within 2 year and between 30-39 years (exposed, cases=15, controls=5, OR=3(2.0-4.0)).
	Deapen DM, 1986, USA	Case control	1. Across USA by mailing to all patients who voluntarily contacting to ALS society of USA or clinicians in private or public clinics to distribute the questionnaires to patients with the disease since August 1977. By March of 1977, 1643 patients returned the questionnaires. The 792 patients still living were mailed to a new questionnaires to collect the information about the risk factor, and provide a list of potential controls who were acquaintances to the patients prior to the diagnosis, and at same gender and age (with 5 years). 2.Of the 792 patients, 678 completed the questionnaires. For control, of the 678 contacted, 518 returned the questionnaires.	1. 518 cases, 518 controls 2. 65% are males, 98% are white, 53.3 years old at average. Similar to the remaining 1125 patients (61%, 96%, and 56.1 years respectively)	Trauma Other cases of unconsciousness 3 y prior to diagnosis: 60 in P, 38 in C, OR=1.6(1.0-2.4) Other cases of unconsciousness 10 y prior to diagnosis: OR=1.6(1.1-2.6) If the episodes of less than 5 minutes were excluded, the difference was diminished: OR=1.5(0.8-2.7); in fact, increasing unconsciousness duration is inversely correlated with risk. No related to surgical trauma which might affected spine.
	Gallagher JP, 1987, USA Florida	Case control	1. Only patients whose disease started before the age of 45, and who have been examined by two or more neurologists, with motor neuron disease diagnosis. 2. They were mailed with questionnaires 3. Response rate: mailed to 181 patients, 135 responded (74%). Controls: MS patients. Sent out to 144 MS patients, 85 responded (59%). Note: MS were better controls.	No such information was provided.	Head Trauma-fracture-strenuous-pneumatic tool 1. Head/neck trauma (yes/total): 31/135, 13/85 2. Fracture of upper extremity: 17/135, 2/85 3. Shoulder and other physical injuries alone: 15/135, 6/85 4. Prior use of pneumatic tools: 14/135, 0/85
	GAWEL M, 1983,UK	Case control	1. 63 patients had been diagnosed as motor neuron disease by one of the study neurologists. A questionnaire 2.61 controls were matched as nearly as possible for age and sex. 3. Self-reported	1. Mean age of the patients was 52.1 years (M 47.5, F 56.7). 2. Mean age of the controls was 53.5 years (M 50.4, F 57.1). 3. Occupation distribution between two groups (manual, professional, active, clerical	Trauma: Injuries to the back were found more frequently in patients as compared to the controls. On the other hand, fractures and head injuries were found less frequently in the patients than in the controls. Forty-two controls had a history of fractures or head injuries in the previous 5 years as opposed to 32 patients. Injury to back (not including fracture and head types of injury, no/injury): <=

				sedentary)was not significantly different.	5yrs, cases=6/57; control=1/61. > 5years: cases=24/39, controls=10/51 (p<0.01). OR for <=5 years: 6.42(4.27-8.57); OR for > 5 years: 3.14 0.85(2.29-3.99).
	Gresham L, 1986, USA	Case control	1. Identified 76 ALS cases, 66 cases participated. 2. Identified patients' neighbour/friends: 66, all participated 3. 50% were men in both groups. 4. Recruited from 01/1985-05/1985	1. Mean age(year, SD, range): 59(sd=11.70, 26-84) in cases, 61.5(sd=11.40, 31-83) 2. 97% were white in both groups 3. There was no difference with regard to twin status, military service, marital status, education levels. 4. Did not mention smoking and alcohol use etc life style related factors.	Trauma (including head, neck, spine, extremities), OR was recalculated. Cases (66 subjects) control(66 subjects) Male female Male female Totals 15 23 17 25 trauma: exposed/unexposed: cases=38/28, CTL=42/24, OR=0.76(0.06-1.46)
	Kondo K, 1981, Japan	Case control	1. Cases were identified from multiple sources, including death certificates. 2. Spouses were served as controls 3. Spouses who have lived with patients since their marriages provided information. If patient who had no spouse to provide this information, or unmarried patients were excluded 4. Spouses were interviewed by a visiting nurse for the events from marriage to the disease onset (not diagnosis) Study B, case-control, age, sex, and residence matched, but the patients and controls were interviewed by neurologists in study B.	1. Cases: 458 men, 254 women. Controls, their wives, or husbands. 1965-1966 2. Study B:1973, 158 cases, sex, age, residence matched control. 104 men, 54 women.	Trauma Study A: 1. Mechanical injuries(to use the raw data, data have to be standardized, because the sex ratio is not balanced): RR=1.74, 35.8% (yes/no=164/288)in male cases, 19.7% (yes/no=90/368)in male controls. RR=2.68, 20.4% (52/202) in female cases, 6.1%(15/239) in female controls. 2. The percentage of every site injury is higher in both men and women cases. both sexes, cases(yes/no)=216/712, controls=105/712. Study B: 1. RR=1.36. for males(yes/no)=36/68, 34.7% in cases, 21.8% (yes/no)=23/71 in controls. RR=1.54 for females. 23.1% (yes/no=12/42)for cases, 9.6% (yes/no=5/49)for controls. For both sex(yes/no), cases=48/110, controls=28/120, OR=1.87(1.34-2.40) Study B: injuries in cases before onset: 0-4y, 14; 6-19y, 16; >20y, 8; injuries in controls before onset: 0-4y, 3; 6-19y, 11; >20y, 8
	Murros K, 1983, Finland	Case control	During the years 1976-81 a total of 43 patients. 4 controls matched by age (+ 2 years), sex and domicile (urban or rural) were chosen for each ALS patient from the files of the Central Hospital. A questionnaire including items about living conditions, earlier diseases, accidents and exposure to domestic animals was sent to the patients (in case of death to a near relative) and to the 172 controls. 88% and 85% respectively, returned a completed questionnaire.	15 men, 21 women, age range between 40-69 for men, 30-79 for women. No race data.	Trauma-fracture 43 cases 172 controls (followings are recalculated results) 1. Injuries (exposed/unexposed): Pa=20/23, CTL=55/117, OR=1.85(1.17-2.53). 2. Fractures (exposed/unexposed): Pa=10/33, CTL=36/136, OR=1.14(0.34-1.94).
	Pierce-Ruhland, 1981, USA	Case control	1. 88 living patients were identified from hospital records. 80 patients participated the study. 2. Patients friends, same sex, age matched with 5 years were suggested by the patients. 80 controls were contacted, 78 controls participated.	1. 80 cases: 53 men, 27 women 2. 78 controls: 52 men, 26 women. 3. Mean age at interview for both groups were the same---52 years. 4. No race data	Trauma Injuries within 10 years of onset reported (exposed/unexposed): Pa=37/43, CTL=28/58, OR=1.78(1.15-2.41).
	Pupillo E, 2012, Italy	Case-control	Cases were patients with newly diagnosed ALS from four population-based registries in Italy. 377 neurological patients and 377 normal controls were selected, matched for age, sex and province of residence.	Need full article to extract the characteristics. Traumatic events (defined as accidental events causing injuries requiring medical care) were recorded with details on type, site, timing, severity, and complications.	Trauma (each group consists of 377 subjects) 1. 225 cases (59.7%), 191 neurological controls (50.7%), and 179 non-neurological controls (47.5%) (P < 0.01) (OR=1.63(1.25-2.14), P < 0.01). 2. OR=3.07 (1.86-5.05) for patients reporting 3+ traumatic events. 3. OR=2.44 (1.36-4.40) for severe traumatic events. 4.The ORs remained significant when the analysis was limited to events occurred 5+ and 10+ years before ALS onset, to incident ALS, and direct informant. 5. Old trauma: 173 cases, 159 neurological controls, 151 non-neurological

					controls suffered before. 6. Fracture: all fractures: 133 in cases, 99 in NC controls, 108 in Non NC controls. 7. Old fractures: 100 in cases, 75 in NC, 86 in NNC. 8. Head injuries: All. 58 in cases, 50 in NC, 38 in NNC. 9. Old head injuries: 40 in cases, 28 in NC, 32 in NNC. 10. Physical activity(occupational): Mild: cases=101 (26.8), NC= 126 (33.4), NNC=131 (34.7) Moderate: cases=123 (32.6) NC=120 (31.8), NNC= 106 (28.1) Strong: cases=153 (40.6) NC=131 (34.7), NNC=140 (37.1) 11.Physical activities(leisure/sport): Yes/all: cases=233/377, NC=241/377, NNC=271/377.
	Savettieri G, 1991, Italy	Case control	Not described for recruitment, no response rate mentioned, study period was not included. Controlled was age, sex, social economic status, living place matched	No further information 46 cases, 92 age, sex, residence place, and social economic status matched	Trauma Trauma(exposed/non-exposed): cases=15/31, controls=17/75, OR=2.30(0.9-6.1)
	Strickland D, 1996, USA, Minnesota	Case control	1. Selected from Mayo clinic. 25 cases were selected. Patients with severe mobility and communication limitations were excluded. 2. Clinic control was selected from same clinic with neuromuscular disease, gender and age matched 2:1. 3. Community control: First 5 digital phone number matched, and gender and age matched. 2:1 4. They were mailed a set of interview forms to help them to prepare and recall question. 5. They were asked to come to the clinic to be interviewed by a nurse interviewer	1. 25 cases, 25 clinic controls, and 25 community controls 2. Males/females proportion was 52%. Average ages was 56.2y in cases, 55.2 in clinic controls, and 56.1 in community controls. Age range is 29-81	Trauma strenuous work sports 1. Trauma (head, neck, back severe injury): 40% (10 subjects) in cases, 20% (5 subjects)in clinic controls, 16% (4 subjects) in community controls. OR=5.3(1.7-17.0). Cases who answered yes were younger than controls (35Y, 39Y and 47Y respectively). 2. Strenuous work (Sweating): OR=1.6(1.1-2.4). OR=3.1(1.04-9.30) 3. for high school organized sports; OR=3.4(1.1-9.9) for organized sports at childhood or adolescence
Old Trauma	Chio A, 1991, Italy	Case control	1. Cases =512 were identified from 1960-1982 from a hospital record consecutive patients. Familiar cases were excluded. 2. Controls=512 with various types of diseases, but not ALS. Admitted into this hospital, admission time, sex, age within 10 years, and residence, matched.	Demographic factors (sex, age, marital status) were similar in both groups	Trauma 1. The number of Trauma in spinal column/upper/lower limbs, and trunk but not head, occurred is higher in patients. 1.1. Total mechanical injuries: cases=113, control=72, OR=1.6(1.3-1.9). 1.2. Frequency of injuries is higher in ALS patients, before diagnosis within 2 year and between 30-39 years (exposed, cases=15, controls=5, OR=3(2.0-4.0)).
	GAWEL M, 1983,UK	Case control	1. 63 patients had been diagnosed as motor neuron disease by one of the study neurologists. A questionnaire 2.61 controls were matched as nearly as possible for age and sex. 3. Self-reported	1. Mean age of the patients was 52.1 years (M 47.5, F 56.7). 2. Mean age of the controls was 53.5 years (M 50.4, F 57.1). 3. Occupation distribution between two groups (manual, professional, active, clerical sedentary)was not significantly different.	Trauma: Injuries to the back were found more frequently in patients as compared to the controls. On the other hand, fractures and head injuries were found less frequently in the patients than in the controls. Forty-two controls had a history of fractures or head injuries in the previous 5 years as opposed to 32 patients. Injury to back (not including fracture and head types of injury, no/injury): <= 5yrs, cases=6/57; control=1/61. > 5years: cases=24/39, controls=10/51 ($p<0.01$). OR for <=5 years: 6.42(4.27-8.57); OR for > 5 years: 3.14 0.85(2.29-3.99).
	Kondo	Case	1. Cases were identified from multiple sources,	1. Cases: 458 men, 254 women. Controls, their	Trauma

	K, 1981, Japan	control	including death certificates. 2. Spouses were served as controls 3. Spouses who have lived with patients since their marriages provided information. If patient who had no spouse to provide this information, or unmarried patients were excluded 4. Spouses were interviewed by a visiting nurse for the events from marriage to the disease onset (not diagnosis) Study B, case-control, age, sex, and residence matched, but the patients and controls were interviewed by neurologists in study B.	wives, or husbands. 1965-1966 2. Study B:1973, 158 cases, sex, age, residence matched control. 104 men, 54 women.	Study A: 1. Mechanical injuries(to use the raw data, data have to be standardized, because the sex ratio is not balanced): RR=1.74, 35.8% (yes/no=164/288)in male cases, 19.7% (yes/no=90/368)in male controls. RR=2.68, 20.4% (52/202) in female cases, 6.1%(15/239) in female controls. 2. The percentage of every site injury is higher in both men and women cases. both sexes, cases(yes/no)=216/712, controls=105/712. Study B: 1. RR=1.36. for males(yes/no)=36/68, 34.7% in cases, 21.8% (yes/no)=23/71 in controls. RR=1.54 for females. 23.1% (yes/no=12/42)for cases, 9.6% (yes/no=5/49)for controls. For both sex(yes/no), cases=48/110, controls=28/120, OR=1.87(1.34-2.40) Study B: injuries in cases before onset: 0-4y, 14; 6-19y, 16; >20y, 8; injuries in controls before onset: 0-4y, 3; 6-19y, 11; >20y, 8
	Pupillo E, 2012, Italy	Case-control	Cases were patients with newly diagnosed ALS from four population-based registries in Italy. 377 neurological patients and 377 normal controls were selected, matched for age, sex and province of residence.	Need full article to extract the characteristics. Traumatic events (defined as accidental events causing injuries requiring medical care) were recorded with details on type, site, timing, severity, and complications.	Trauma (each group consists of 377 subjects) 1. 225 cases (59.7%), 191 neurological controls (50.7%), and 179 non-neurological controls (47.5%) (P < 0.01) (OR=1.63(1.25-2.14), P < 0.01). 2. OR=3.07 (1.86-5.05) for patients reporting 3+ traumatic events. 3. OR=2.44 (1.36-4.40) for severe traumatic events. 4.The ORs remained significant when the analysis was limited to events occurred 5+ and 10+ years before ALS onset, to incident ALS, and direct informant. 5. Old trauma: 173 cases, 159 neurological controls, 151 non-neurological controls suffered before. 6. Fracture: all fractures: 133 in cases, 99 in NC controls, 108 in Non NC controls. 7. Old fractures: 100 in cases, 75 in NC, 86 in NNC. 8. Head injuries: All. 58 in cases, 50 in NC, 38 in NNC. 9. Old head injuries: 40 in cases, 28 in NC, 32 in NNC. 10. Physical activity(occupational): Mild: cases=101 (26.8), NC= 126 (33.4), NNC=131 (34.7) Moderate: cases=123 (32.6) NC=120 (31.8), NNC= 106 (28.1) Strong: cases=153 (40.6) NC=131 (34.7), NNC=140 (37.1) 11.Physical activities(leisure/sport): Yes/all: cases=233/377, NC=241/377, NNC=271/377.
Bone fracture	Chancellor AM, 1993, England	Case control	1. Of 147 such patients diagnosed between 1 May 1990 and 31 October 1991, 39 had died before approach was possible. 2. Controls (paired case-control study. Controls were identified by GP. Controls with dementia were excluded) were selected by sex, age(as close as possible), living placed matched	The age and sex of cases and controls were identical, a result of the matching process. There were 61 men (mean age 63, range 35-85 years) and 42 women (mean age 67 range 28-86 years).	Fracture 1. Total fractures were associated with ALS only in females: Male, OR=0.7(0.3-1.7); Females, OR=2.8(1.1-8.8); both OR=1.3(0.7-2.5) 2. But this this association might be mainly attributable to the occurrence of trauma with 5 years before MND diagnosis: males OR>1; Females, OR=8 (1.1-348); both, OR=15 (2.3-654) 3. Fracture number: <5 years(fractures): cases(15/88), control(1/102) >=5 years(fractures): cases(39/84), controls(45/78)
	Cruz DCN, 1999, USA	Case control	1. From 1990-1994, 180 incident ALS patients were identified in 4 Western Washington state (4 counties). 174 agreed to participated this studies. 20 case patients died before the interviewed, but the information was provided by surrogates. 2. Two controls were selected for each by	1. males/females=95/79 2. White/non=163/11 3. Age range 18->75; close to controls, marital and educations were also close	Fracture: Fracture: not associated (any fracture, occurred <=5, 6-20, >20 years) Any fracture(yes/no): cases=89/85. controls=188/160, OR=0.9(0.6-1.3)

			matching gender and sex within 5 years. First cohort of controls was selected by random telephone dialling, 221 out of 267 eligible controls agreed to participated. Second cohort was selected from medical care eligibility. 121 out 202 controls agreed to participate.		
	Gallagher JP, 1987, USA Florida	Case control	1. Only patients whose disease started before the age of 45, and who have been examined by two or more neurologists, with motor neuron disease diagnosis. 2. They were mailed with questionnaires 3. Response rate: mailed to 181 patients, 135 responded (74%). Controls: MS patients. Sent out to 144 MS patients, 85 responded (59%). Note: MS were better controls.	No such information was provided.	Head Trauma-fracture-strenuous-pneumatic tool 1. Head/neck trauma (yes/total): 31/135, 13/85 2. Fracture of upper extremity: 17/135, 2/85 3. Shoulder and other physical injuries alone: 15/135, 6/85 4. Prior use of pneumatic tools: 14/135, 0/85
	Kihira T, 2007, Japan	Case control	1. Consecutively admitted in to Wkayama Medicla Hosapital from 1999-2004 2. The population in the prefecture is about 1 million. more 20% are 65 or more years old. 3. This Hospital has diagnosed about 50% of ALS patients in this Prefecture 108 ALS patients 302 neurological patient controls, 40 or older were recruited	1. 108 patients (definite and probable), 67 males, 41 females. Average age is 61.5±10.4 for both sexes. 2. 244 controls: 146 males, 156 females. Age is at 62.1±10.8 and 64.0±9.9 for males and females respectively	Fracture-strenuous work 1. Past bone fracture: ALS, 22.2%, control, 11.6%. OR=2.1 (1.12-3.94) 2. Primary industry(agriculture, forestry, fishing): 254 controls, 43 ALS: control(9.50%), ALS at 23.10%, OR=2.69 (1.40-5.16). 3. Secondary industry(ironwork, construction, chemical handling, traffic, transportation):control, 244 subjects, ALS, 53 subjects: Control at 12.7%, ALS at 26.90%, OR=2.81(1.45-5.46) 4.Tertiaryindustries(service, office, business): control, 214 subjects, ALS, 83 subjects: Control at 31.7%, ALS at 21.30%, OR=0.54(0.30-0.98).
	Murros K, 1983, Finland	Case control	During the years 1976-81 a total of 43 patients. 4 controls matched by age (+ 2 years), sex and domicile (urban or rural) were chosen for each ALS patient from the files of the Central Hospital. A questionnaire including items about living conditions, earlier diseases, accidents and exposure to domestic animals was sent to the patients (in case of death to a near relative) and to the 172 controls. 88% and 85% respectively, returned a completed questionnaire.	15 men, 21 women, age range between 40-69 for men, 30-79 for women. No race data.	Trauma-fracture 43 cases 172 controls (followings are recalculated results) 1. Injuries (exposed/unexposed): Pa=20/23, CTL=55/117, OR=1.85(1.17-2.53). 2. Fractures (exposed/unexposed): Pa=10/33, CTL=36/136, OR=1.14(0.34-1.94).
	Okamoto K, 2009, Japan	Case control	1. Of the 274 ALS patients, 214 (75.3%) eligible cases were enrolled, 183 of whom completed the entire questionnaire. 2.Two community controls that matched to each case for age (G2 years) and gender. 3. Among a total of 732 eligible controls contacted, 550 (75.2%) were enrolled in this study and 430 completed the entire questionnaire.	1. From 2000 to 2005 2. Cases (n 153), Controls (n 306), 60.3% men for cases and controls. 3. Mean age (G SD) 63.7±9.2 for cases 63.4±10.6 controls.	Fracture-sports: 1. Fracture: 19.6% for cases, 15.1% for control, OR=1.3 (0.9–2.1). 2. Vigour activity: 11.6% for cases, 6.2% for control OR=2.0 (1.0–4.0)
	Provinciali L, 1990, Italy	Case control	1. 77 patients (57 males, 20 females, mean age=59±8) from 89 consecutive patients from a clinic during 1979-1987. 2. Controls (80) with various types of neuron diseases (including infectious diseases)were	No race information.	Fracture--strenuous work- hard work 1. Hard labor(exposed/non-exposed): Pa=54/23, CTL=39/41, OR=2.47(1.74-3.13). 2. trauma(fractures/no): Pa=19/58, CTL=12/68, OR=1.86(1.06-2.66)

			matched by age, sex, life-style(alcohol drinking, smoking), education, origin, cultural background from in patients over same period		
	Pupillo E, 2012, Italy	Case-cotrol	Cases were patients with newly diagnosed ALS from four population-based registries in Italy. 377 neurological patients and 377 normal controls were selected, matched for age, sex and province of residence.	Need full article to extract the characteristics. Traumatic events (defined as accidental events causing injuries requiring medical care) were recorded with details on type, site, timing, severity, and complications.	Trauma (each group consists of 377 subjects) 1. 225 cases (59.7%), 191 neurological controls (50.7%), and 179 non-neurological controls (47.5%) (P < 0.01) (OR=1.63(1.25-2.14), P < 0.01). 2. OR=3.07 (1.86-5.05) for patients reporting 3+ traumatic events. 3. OR=2.44 (1.36-4.40) for severe traumatic events. 4.The ORs remained significant when the analysis was limited to events occurred 5+ and 10+ years before ALS onset, to incident ALS, and direct informant. 5. Old trauma: 173 cases, 159 neurological controls, 151 non-neurological controls suffered before. 6. Fracture: all fractures: 133 in cases, 99 in NC controls, 108 in Non NC controls. 7. Old fractures: 100 in cases, 75 in NC, 86 in NNC. 8. Head injuries: All. 58 in cases, 50 in NC, 38 in NNC. 9. Old head injuries: 40 in cases, 28 in NC, 32 in NNC. 10. Physical activity(occupational): Mild: cases=101 (26.8), NC= 126 (33.4), NNC=131 (34.7) Moderate: cases=123 (32.6) NC=120 (31.8), NNC= 106 (28.1) Strong: cases=153 (40.6) NC=131 (34.7), NNC=140 (37.1) 11.Physical activities(leisure/sport): Yes/all: cases=233/377, NC=241/377, NNC=271/377.
Head injury	Beghi E, 2010, Italy, UK, and Ireland	Case control	1. P: have to be definite, probable, or possible ALS cases, did mention when the study was done 2. Age (±2.5 y) and sex matched for control for each patient, selected by general practitioners for the patients 3. Report response rate is more than 90%, no further data	1. Cases/controls=61/112. P, 27 F, 34 M; C, 46 F, 66 M, not exactly matched. 2. Age, marital, education, no difference between P and C.	Trauma: head, sport, strenuous workers, professional sports 1. All trauma (yes/no), no difference: P=29/32, C=59/53 2. Head trauma (yes/no): no difference: P, 4/57; C, 11/101 3. Repeated trauma (yes/no): P, 21/40; C, 27/85, OR=1.65(0.97-2.33) 4. Work intensity (Yes/no, P<0.05): Strenuous/all, P=8/53, C=4/108; 5. Sport intensity (yes/no, P>0.05): strenuous/all others, p=8/53, C=19/93 6. Professional sport(yes/no, p=0.04):P=3/58, C=0/112 BMI BMI. cases: 25.3, SD 4.5; controls: 26.1, SD 4.1 Details were not provided in this paper
	Binazzi A, 2009; Italy	Case control	1. Recruited since 17J, 2005 to July 6, 2007. 77 patients, 70 definite, 7 probable. 44 spinal/ 32 bulbar 2. Relatives or accompanying persons of outpatients affected by neurological disease other than ALS, and coming to the same hospital ambulatories, service as population controls 3. No response rate was mentioned	1. Cases, 77; 43, males, 34 females, 44 spinal, 32 bulbar. age=65±9.3, range 42-83. onset ages: 62.4(60.1-64.7), age at diagnosis, 63.7(61.4-66.1). 2. Control: 185 (male=69, females=116), different to cases. Average=57.5±13.0, 28-84.	Trauma-head, leg-strenuous workers 1.Head injury: Years since last injuries: 11-30y, bulbar, OR=3.51 (1.03-11,95), not spinal, not <=10y, or >30y with significance. 2. Ages at last injury: <30y, spinal, OR=7.13(1.34-37.94); 30-40y, bulbar, OR=17.4(1.70-178.5), not other age groups, or onset conditions. 3. Leg injury: OR=1.82(1.01-3.29) for all cases (19/58, control=31/154), other sites, or any site, or separate calculation for bulbar, spinal, no difference. 4. Building or construction: spinal, OR=3.01(1.01-8.99, 6/38; C=6/179); not bulbar, not all cases. 5. Exposed to building and construction materials: spinal, OR=5.27(1.15-24.12, 4/40, C=3/182)
	Chen, H;	Case control	1. Recruitment time and place: 1993-1996 from two major referral centers in New England.	1. Sample size: patients: 109/control:255 2. Race and sex ratio might be in their	Trauma-head injury-total injury 1. Head injury: OR=1.4 (0.8-2.8), cases: 22.0 per cent, 16.5% in control

	2007; USA, Boston		2. Has been diagnosed within 2 years, lived in New England for at least 50% of the years, spoke English, mentally competent. Control: Random selected via telephone screening, no neurological disease, gender match, age match, at 3:1 for Boston area, and 2:1 for other areas. 3. response rate: patients: 110/154 (two could not be found, 42 declined(7 due to illness, 15 due to travelling difficulties, 20 for other reasons)), 1 missing, final=109 Control: 256 competed questionnaires from 354 contacted, 1 missing, final=255	previous papers. 3. Divided patients into three age groups (30-55, 56-65, 66-80) 4. Study time: 1993-1996 5. Average age at diagnosis, 58.3 y; from self-reported sign to diagnosis is 14.3 months, 27/bulbar, 82 limb onset	2. Total injuries: OR=1.2(0.7-1.9), cases, 46.8%, 45.1% in control 3. 1 head injury: OR=0.9(0.4-2.0), cases=11.9%, control=12.5%; >1, OR=3.1(1.2-8.1), p=10.1%, C=3.9%. 4. Years since last injury to ALS diagnosis: <=10, OR=3.2(1.0-10.2), P=7.3%, c=2.4%; 11-30, 1.2(0.5-2.9, p=10.1%, c=8.2%; >30, OR=0.9(0.3-2.7), p=4.6%, c=5.9%. 5. Exclude cases with family history, and the injuries within 3 years from reference date (diagnostic date in cases or 2 years before the interview date), within past 10 years: OR=5.6 (1.3-24.4), no further data/number available.
	Furby, J 2010, France	Case control	1. This is a rural area with a rather stable population of about three million inhabitants. All subjects had been living in Brittany for more than 1 year. 2. 2006–2008. 3. The 108 patients, 122 controls were enrolled consecutively from the orthopedic service of Saint-Brieuc Hospital where they had been hospitalized for minor traumas. Controls were age and sex-matched to the patients. Within the control group, all subjects with chronic neurological disease or alcohol consumption were excluded.	Patients Controls Age at interview M=68 SD=18.0 range[34–86] M=65; SD=18.0 range[31–84] Sex men/women 59/49 68/54	Trauma-strenuous work 1. head trauma Cases control OR Head injuries yes/no (reference) 19/89 14/108 1.859 [0.861–4.011] p=0.114 2. Strenuous work ALS control OR Teaching professionals 2 10 0.205 [0.042–0.990], P=0.049 Market-oriented skilled agricultural workers 22 10 2.884 [1.274–6.530], p=0.011 Metal, machinery and related trade workers 5 2
	Gallagher JP, 1987, USA Florida	Case control	1. Only patients whose disease started before the age of 45, and who have been examined by two or more neurologists, with motor neuron disease diagnosis. 2. They were mailed with questionnaires 3. Response rate: mailed to 181 patients, 135 responded (74%). Controls: MS patients. Sent out to 144 MS patients, 85 responded (59%). Note: MS were better controls.	No such information was provided.	Head Trauma-fracture-strenuous-pneumatic tool 1. Head/neck trauma (yes/total): 31/135, 13/85 2. Fracture of upper extremity: 17/135, 2/85 3. Shoulder and other physical injuries alone: 15/135, 6/85 4. Prior use of pneumatic tools: 14/135, 0/85
	Pupillo E, 2012, Italy		Cases were patients with newly diagnosed ALS from four population-based registries in Italy. 377 neurological patients and 377 normal controls were selected, matched for age, sex and province of residence.	Need full article to extract the characteristics. Traumatic events (defined as accidental events causing injuries requiring medical care) were recorded with details on type, site, timing, severity, and complications.	Trauma (each group consists of 377 subjects) 1. 225 cases (59.7%), 191 neurological controls (50.7%), and 179 non-neurological controls (47.5%) ($P < 0.01$) (OR=1.63(1.25-2.14), $P < 0.01$). 2. OR=3.07 (1.86-5.05) for patients reporting 3+ traumatic events. 3. OR=2.44 (1.36-4.40) for severe traumatic events. 4.The ORs remained significant when the analysis was limited to events occurred 5+ and 10+ years before ALS onset, to incident ALS, and direct informant. 5. Old trauma: 173 cases, 159 neurological controls, 151 non-neurological controls suffered before. 6. Fracture: all fractures: 133 in cases, 99 in NC controls, 108 in Non NC controls. 7. Old fractures: 100 in cases, 75 in NC, 86 in NNC. 8. Head injuries: All. 58 in cases, 50 in NC, 38 in NNC.

					9. Old head injuries: 40 in cases, 28 in NC, 32 in NNC. 10. Physical activity(occupational): Mild: cases=101 (26.8), NC= 126 (33.4), NNC=131 (34.7) Moderate: cases=123 (32.6) NC=120 (31.8), NNC= 106 (28.1) Strong: cases=153 (40.6) NC=131 (34.7), NNC=140 (37.1) 11.Physical activities(leisure/sport): Yes/all: cases=233/377, NC=241/377, NNC=271/377.
	Schmidt S, 2010, USA	Case control	1. Recruitment: active, US veterans between April 2003 and September 2007; passive, based on multiple ad and publicity method, excluded patients with family history. 2. Frequency-matched control, age, sex, absence of ALS and other neurological diseases. 3. 6.5% re*FUS*al rate for active recruitment, >1% for self-reported cases, for control, 41% of eligible controls had consented to participated	1. P, 241 cases; C, 577. 2. Male: 97.9% in cases; 94.1% in control 3. Age: 62.4, SD=10.3; 61.7%, SD=10.6 4. Race: 94.6% non-Hispanic white in cases, 88.4% in control	Trauma Head injury: Table 2 and Fig 1. 1. age at last injury: >=29, OR=1.88(1.15-3.08), other age group, no difference. 2. Years between last injury to reference date: 2-5 years: OR=1.62(0.52-5.28) 6-10 years: OR=2.28(0.59-8.88) 11-15 years: OR=3.24(1.15-9.12) 2-15 years: OR=2.33(1.18-4.61) >15 years: OR=1.03(0.72-1.47)
	*Turner MR, 2010, UK	Cohort Study	Data linkage from existing datasets in England. Oxford Record Linkage Study (ORLS), which spans a period from January 1963 to March 1999.	Three populations: from 1963 to March 1999 (350000) From 1966 to March 1999 (800000) From 1975 to March 1999 (1.9million) From 1987 to March 1999 (2.5million) Can not find how many cases identified.	trauma-head limb observed / expected / ratio Head ulimb Llimb total (each) 0-1: 14 8 17 39 / 4.6 3.1 7.1 14.8 / 4.6 3.3 3.7 2.6 1-4: 15 11 23 49 / 11 9.4 21.7 32.1 / 1.4 1.2 1.1 1.5 5-9: 9 7 15 31 / 7.6 7 11.7 26.3 / 1.2 1.0 1.4 1.2 10-19: 11 6 5 22 / 10.1 6.1 7.4 23.6 / 1.1 1.0 0.7 0.9 20+: 6 5 1 12 / 5.2 2 1.9 9.1 / 1.2 2.9 0.5 1.3 total: 55 37 61 143 / 38.6 27.7 49.9 116.2 / 1.5 1.4 1.3 1.2
	*Qureshi MM, 2006,USA	Case control	1. Between April 1998 and August 2002, recruited 95 subjects with ALS and 106 healthy control subjects in this study. 2. Cases were identified from clinic. 3. Controls were non-blood relative(spouse), friends, unrelated subjects(age matched).At recruitment controls were matched to the ALS subjects by gender and age.	cases Controls Gender – Males 60 (63.2 %) 58 (54.7%) Race – Caucasian 91 (95.8 %) 102 (96.2 %) Mean age at enrolment: 54.4 ± 13.1 (SD) 52.5 ±14.9 (SD) Weight (Kg) – Females 66 ±19 (SD) 65 ±10 (SD) Weight (Kg) – Males 82 ±13 (SD) 86 ±17 (SD)	Trauma-head History of head trauma 0.79 (0.37–1.68), p=0.54
Sports	Beghi E, 2010, Italy, UK, and Ireland	Case control	1. P: have to be definite, probable, or possible ALS cases, did mention when the study was done 2. Age (±2.5 y) and sex matched for control for each patient, selected by general practitioners for the patients 3. Report response rate is more than 90%, no further data	1. Cases/controls=61/112. P, 27 F, 34 M; C, 46 F, 66 M, not exactly matched. 2. Age, marital, education, no difference between P and C.	Trauma: head, sport, strenuous workers, professional sports 1. All trauma (yes/no), no difference: P=29/32, C=59/53 2. Head trauma (yes/no): no difference: P, 4/57; C, 11/101 3. Repeated trauma (yes/no): P, 21/40; C, 27/85, OR=1.65(0.97-2.33) 4. Work intensity (Yes/no, P<0.05): Strenuous/all, P=8/53, C=4/108; 5. Sport intensity (yes/no, P>0.05): strenuous/all others, p=8/53, C=19/93 6. Professional sport(yes/no, p=0.04):P=3/58, C=0/112 BMI BMI. cases: 25.3, SD 4.5; controls: 26.1, SD 4.1 Details were not provided in this paper
	Longst	Case	1. From 1990-1994, 180 incident ALS patients	1. males/females=95/79	Sports

	reth WT, USA year	control	were identified in 4 Western Washington state (4 counties). 174 agreed to participated this studies. 20 case patients died before the interviewed, but the information was provided by surrogates. 2. Two controls were selected for each by matching gender and sex within 5 years. First cohort of controls was selected by random telephone dealing, 221 out of 267 eligible controls agreed to participated. Second cohort was selected from medical care eligibility. 121 out 202 controls agreed to participate.	2. White/non=163/11 3. Age range 18->75; close to controls, marital and educations were also close 4. 174 cases, 348 controls.	1. All overall ORs were not found different, except the participation of organized sports during high school: cases (exposed/unexposed)=(80/94), controls(exposed/unexposed)=131/217; OR= 1.52 (1.03-2.25). 2. The sliding window analysis for various types of activities (workplace, leisure time, during different time periods before the reference date, or against the age of years of the patients), there is no significant period was detected.
	Okamoto K, 2009, Japan	Case control	1. Of the 274 ALS patients, 214 (75.3%) eligible cases were enrolled, 183 of whom completed the entire questionnaire. 2.Two community controls that matched to each case for age (G2 years) and gender. 3. Among a total of 732 eligible controls contacted, 550 (75.2%) were enrolled in this study and 430 completed the entire questionnaire.	1. From 2000 to 2005 2. Cases (n 153), Controls (n 306), 60.3% men for cases and controls. 3. Mean age (G SD) 63.7±9.2 for cases 63.4±10.6 controls.	Fracture-sports: 1. Fracture: 19.6% for cases, 15.1% for control, OR=1.3 (0.9–2.1). 2. Vigour activity: 11.6% for cases, 6.2% for control OR=2.0 (1.0–4.0)
	Pupillo E, 2012, Italy	Case-control	Cases were patients with newly diagnosed ALS from four population-based registries in Italy. 377 neurological patients and 377 normal controls were selected, matched for age, sex and province of residence.	Need full article to extract the characteristics. Traumatic events (defined as accidental events causing injuries requiring medical care) were recorded with details on type, site, timing, severity, and complications.	Trauma (each group consists of 377 subjects) 1. 225 cases (59.7%), 191 neurological controls (50.7%), and 179 non-neurological controls (47.5%) (P < 0.01) (OR=1.63(1.25-2.14), P < 0.01). 2. OR=3.07 (1.86-5.05) for patients reporting 3+ traumatic events. 3. OR=2.44 (1.36-4.40) for severe traumatic events. 4.The ORs remained significant when the analysis was limited to events occurred 5+ and 10+ years before ALS onset, to incident ALS, and direct informant. 5. Old trauma: 173 cases, 159 neurological controls, 151 non-neurological controls suffered before. 6. Fracture: all fractures: 133 in cases, 99 in NC controls, 108 in Non NC controls. 7. Old fractures: 100 in cases, 75 in NC, 86 in NNC. 8. Head injuries: All. 58 in cases, 50 in NC, 38 in NNC. 9. Old head injuries: 40 in cases, 28 in NC, 32 in NNC. 10. Physical activity(occupational): Mild: cases=101 (26.8), NC= 126 (33.4), NNC=131 (34.7) Moderate: cases=123 (32.6) NC=120 (31.8), NNC= 106 (28.1) Strong: cases=153 (40.6) NC=131 (34.7), NNC=140 (37.1) 11.Physical activities(leisure/sport): Yes/all: cases=233/377, NC=241/377, NNC=271/377.
	Vanacore N, 2010, USA	Case control	U.S. mortality database 1. Cases: 14,628 deaths due to ALS 2. Controls: 58,512 controls from other selected causes of death, frequency matched by age, gender and broad geographic area	Cases:14628 Controls: 58512	Physical activity at work 1. Professional athletes: Cases 6, controls 13, OR=1.81 0.50–6.77 2. Physical activity at work∗ moderate: Males: Cases 1787, controls 7087, OR=0.94 (0.87–1.03) Females: cases 3984, controls 17350, OR=0.79 (0.73–0.85) intense: Males: cases 1841, controls 9030, OR=0.95 (0.86–1.04) Females: cases 318, controls 1350, OR=1.00 (0.82–1.20)
	Veldin	Case	1. All patients included in this study were	1. Age match was good (mean=59 for both	Sports

	k JH, 2005 Netherlands	control	incident cases who visited clinics for diagnostic purposes during the 1-year period 2001 to 2002. 2. 280 patients with sporadic ALS were identified and were sent a questionnaire, 219 of which were returned (78%). 3. Controls were approached by patients according to sex, age criteria. Did not give response rate.	patients and controls), but not sex ratio is different (men/women, cases= 146/73; controls=145/109, P=0.03). 2. Lifestyle also matched, but not optimal: Education(p=0.66) BMI(p=0.54) alcohol(p=0.07) smoking(p=0.08)	1. CASES(%) Control(%) OR(95%CI) Pvalue Engaged in sports as youngster* 142 (67) 162 (66) 1.0 (0.6–1.5) 0.85 Engaged in sports as adult† 160 (76) 182 (74) 1.3 (0.8–2.1) 0.36 Ever extreme physical activity‡ 25 (12) 25 (11) 1.2 (0.6–2.3) 0.59 2. Divided sports activity into quartiles from professional and amateur two groups. Some positive trends were observed, but not significant.
	*Valenti M, 2005, Italy	Case control	1. Subjects were 300 new consecutive cases of probable or definite ALS, at 10 national reference centers. 2. from January 2002 to May 2003.	. There were 193 males (mean age 59 ± 8 years, range 32–73) and 107 females (mean age 60 ± 9 years, range 40–73). Nine familial ALS (FALS) patients were included, and in three of them SOD-1 mutations were identified.	Sports trauma 1. Amateur practice of soccer, competitive practice of soccer, Amateur practice of sports other than soccer, competitive practice of sports other than soccer, Sports-related trauma are negatively associated with ALS
Organized sports	Beghi E, 2010, Italy, UK, and Ireland	Case control	1. P: have to be definite, probable, or possible ALS cases, did mention when the study was done 2. Age (±2.5 y) and sex matched for control for each patient, selected by general practitioners for the patients 3. Report response rate is more than 90%, no further data	1. Cases/controls=61/112. P, 27 F, 34 M; C, 46 F, 66 M, not exactly matched. 2. Age, marital, education, no difference between P and C.	Trauma: head, sport, strenuous workers, professional sports 1. All trauma (yes/no), no difference: P=29/32, C=59/53 2. Head trauma (yes/no): no difference: P, 4/57; C, 11/101 3. Repeated trauma (yes/no): P, 21/40; C, 27/85, OR=1.65(0.97-2.33) 4. Work intensity (Yes/no, P<0.05): Strenuous/all, P=8/53, C=4/108; 5. Sport intensity (yes/no, P>0.05): strenuous/all others, p=8/53, C=19/93 6. Professional sport(yes/no, p=0.04):P=3/58, C=0/112 BMI BMI. cases: 25.3, SD 4.5; controls: 26.1, SD 4.1 Details were not provided in this paper
	Longstreth WT, USA year	Case control	1. From 1990-1994, 180 incident ALS patients were identified in 4 Western Washington state (4 counties). 174 agreed to participated this studies. 20 case patients died before the interviewed, but the information was provided by surrogates. 2. Two controls were selected for each by matching gender and sex within 5 years. First cohort of controls was selected by random telephone dealing, 221 out of 267 eligible controls agreed to participated. Second cohort was selected from medical care eligibility. 121 out 202 controls agreed to participate.	1. males/females=95/79 2. White/non=163/11 3. Age range 18->75; close to controls, marital and educations were also close 4. 174 cases, 348 controls.	Sports 1. All overall ORs were not found different, except the participation of organized sports during high school: cases (exposed/unexposed)=(80/94), controls(exposed/unexposed)=131/217; OR= 1.52 (1.03-2.25). 2. The sliding window analysis for various types of activities (workplace, leisure time, during different time periods before the reference date, or against the age of years of the patients), there is no significant period was detected.
	Pupillo E, 2012, Italy	Case control	Cases were patients with newly diagnosed ALS from four population-based registries in Italy. 377 neurological patients and 377 normal controls were selected, matched for age, sex and province of residence.	Need full article to extract the characteristics. Traumatic events (defined as accidental events causing injuries requiring medical care) were recorded with details on type, site, timing, severity, and complications.	Trauma (each group consists of 377 subjects) 1. 225 cases (59.7%), 191 neurological controls (50.7%), and 179 non-neurological controls (47.5%) (P < 0.01) (OR=1.63(1.25-2.14), P < 0.01). 2. OR=3.07 (1.86-5.05) for patients reporting 3+ traumatic events. 3. OR=2.44 (1.36-4.40) for severe traumatic events. 4.The ORs remained significant when the analysis was limited to events occurred 5+ and 10+ years before ALS onset, to incident ALS, and direct informant. 5. Old trauma: 173 cases, 159 neurological controls, 151 non-neurological controls suffered before. 6. Fracture: all fractures: 133 in cases, 99 in NC controls, 108 in Non NC

					controls. 7. Old fractures: 100 in cases, 75 in NC, 86 in NNC. 8. Head injuries: All. 58 in cases, 50 in NC, 38 in NNC. 9. Old head injuries: 40 in cases, 28 in NC, 32 in NNC. 10. Physical activity(occupational): Mild: cases=101 (26.8), NC= 126 (33.4), NNC=131 (34.7) Moderate: cases=123 (32.6) NC=120 (31.8), NNC= 106 (28.1) Strong: cases=153 (40.6) NC=131 (34.7), NNC=140 (37.1) 11.Physical activities(leisure/sport): Yes/all: cases=233/377, NC=241/377, NNC=271/377.
	Veldink JH, 2005 Netherlands	Case control	1. All patients included in this study were incident cases who visited clinics for diagnostic purposes during the 1-year period 2001 to 2002. 2. 280 patients with sporadic ALS were identified and were sent a questionnaire, 219 of which were returned (78%). 3. Controls were approached by patients according to sex, age criteria. Did not give response rate.	1. Age match was good (mean=59 for both patients and controls), but not sex ratio is different (men/women, cases= 146/73; controls=145/109, P=0.03). 2. Lifestyle also matched, but not optimal: Education(p=0.66) BMI(p=0.54) alcohol(p=0.07) smoking(p=0.08)	Sports 1. CASES(%) Control(%) OR(95%CI) Pvalue Engaged in sports as youngster* 142 (67) 162 (66) 1.0 (0.6–1.5) 0.85 Engaged in sports as adult† 160 (76) 182 (74) 1.3 (0.8–2.1) 0.36 Ever extreme physical activity‡ 25 (12) 25 (11) 1.2 (0.6–2.3) 0.59 2. Divided sports activity into quartiles from professional and amateur two groups. Some positive trends were observed, but not significant.
	*Valenti M, 2005, Italy	Case control	1. Subjects were 300 new consecutive cases of probable or definite ALS, at 10 national reference centers. 2. from January 2002 to May 2003.	. There were 193 males (mean age 59 ± 8 years, range 32–73) and 107 females (mean age 60 ± 9 years, range 40–73). Nine familial ALS (FALS) patients were included, and in three of them SOD-1 mutations were identified.	Sports trauma 1. Amateur practice of soccer, competitive practice of soccer, Amateur practice of sports other than soccer, competitive practice of sports other than soccer, Sports-related trauma are negatively associated with ALS
Strenuous work	Beghi E, 2010, Italy, UK, and Ireland	Case control	1. P: have to be definite, probable, or possible ALS cases, did mention when the study was done 2. Age (±2.5 y) and sex matched for control for each patient, selected by general practitioners for the patients 3. Report response rate is more than 90%, no further data	1. Cases/controls=61/112. P, 27 F, 34 M; C, 46 F, 66 M, not exactly matched. 2. Age, marital, education, no difference between P and C.	Trauma: head, sport, strenuous workers, professional sports 1. All trauma (yes/no), no difference: P=29/32, C=59/53 2. Head trauma (yes/no): no difference: P, 4/57; C, 11/101 3. Repeated trauma (yes/no): P, 21/40; C, 27/85, OR=1.65(0.97-2.33) 4. Work intensity (Yes/no, P<0.05): Strenuous/all, P=8/53, C=4/108; 5. Sport intensity (yes/no, P>0.05): strenuous/all others, p=8/53, C=19/93 6. Professional sport(yes/no, p=0.04):P=3/58, C=0/112 BMI BMI. cases: 25.3, SD 4.5; controls: 26.1, SD 4.1 Details were not provided in this paper
	Binazzi A, 2009; Italy	Case control	1. Recruited since 17J, 2005 to July 6, 2007. 77 patients, 70 definite, 7 probable. 44 spinal/ 32 bulbar 2. Relatives or accompanying persons of outpatients affected by neurological disease other than ALS, and coming to the same hospital ambulatories, service as population controls 3. No response rate was mentioned	1. Cases, 77; 43, males, 34 females, 44 spinal, 32 bulbar. age=65±9.3, range 42-83. onset ages: 62.4(60.1-64.7), age at diagnosis, 63.7(61.4-66.1). 2. Control: 185 (male=69, females=116), different to cases. Average=57.5±13.0, 28-84.	Trauma-head, leg-strenuous workers 1.Head injury: Years since last injuries: 11-30y, bulbar, OR=3.51 (1.03-11,95), not spinal, not <=10y, or >30y with significance. 2. Ages at last injury: <30y, spinal, OR=7.13(1.34-37.94); 30-40y, bulbar, OR=17.4(1.70-178.5), not other age groups, or onset conditions. 3. Leg injury: OR=1.82(1.01-3.29) for all cases (19/58, control=31/154), other sites, or any site, or separate calculation for bulbar, spinal, no difference. 4. Building or construction: spinal, OR=3.01(1.01-8.99, 6/38; C=6/179); not bulbar, not all cases. 5. Exposed to building and construction materials: spinal, OR=5.27(1.15-24.12, 4/40, C=3/182)
	Fang F,	Case	1. All study participants (cases and controls)	1. The median age at diagnosis for cases was	Strenuous-blue collar

	2009, USA	control	were recruited between 1993 and 1996 2. Sequential ALS cases were recruited from two major referral centers in New England. 3. Cases were required to live in New England for at least 50% of the year, to be mentally competent, and to speak English. 71% of eligible cases participated in the study (n = 111). About 85% of the cases were enrolled within 1 year after diagnosis and the remainder within 2 years. 4. Population controls were identified through random telephone screening, no neurological disease, sex, age (30–55, 56–65, and 66–80 years) and region matched. 354 eligible controls were contacted, 270 (76%) were enrolled, 256 completed the entire questionnaire.	60 years (range, 30–79 years) and the median age at 2 years before interview for controls was 59 years (range, 29–78 years). Diagnosis was made 14.3 months after the onset of symptoms on average. 2. Male: Cases, 66 (60.6); controls, 156 (61.7). Female: Cases, 43 (39.4); controls, 97 (38.3) 3. 27 (25%) of the cases had a bulbar onset and 82 (75%) had a trunk or limb onset.	controls Cases OR (95%CI) 1. Administrative support, clerical: 80 42 1.4 (0.9–2.4) 2. Construction trades: 13 14 2.5 (1.0–5.8) 3. Precision production: 21 19 2.2 (1.1–4.4) 4. Transportation/material moving : 16 14 1.9 (0.9–4.3)
	Gallagher JP, 1987, USA Florida	Case control	1. Only patients whose disease started before the age of 45, and who have been examined by two or more neurologists, with motor neuron disease diagnosis. 2. They were mailed with questionnaires 3. Response rate: mailed to 181 patients, 135 responded (74%). Controls: MS patients. Sent out to 144 MS patients, 85 responded (59%). Note: MS were better controls.	No such information was provided.	Head Trauma-fracture-strenuous-pneumatic tool 1. Head/neck trauma (yes/total): 31/135, 13/85 2. Fracture of upper extremity: 17/135, 2/85 3. Shoulder and other physical injuries alone: 15/135, 6/85 4. Prior use of pneumatic tools: 14/135, 0/85
	Kihira T, 2007, Japan	Case control	1. Consecutively admitted in to Wkayama Medicla Hosapital from 1999-2004 2. The population in the prefecture is about 1 million. more 20% are 65 or more years old. 3. This Hospital has diagnosed about 50% of ALS patients in this Prefecture 108 ALS patients 302 neurological patient controls, 40 or older were recruited	1. 108 patients (definite and probable), 67 males, 41 females. Average age is 61.5±10.4 for both sexes. 2. 244 controls: 146 males, 156 females. Age is at 62.1±10.8 and 64.0±9.9 for males and females respectively	Fracture-strenuous work 1. Past bone fracture: ALS, 22.2%, control, 11.6%. OR=2.1 (1.12-3.94) 2. Primary industry(agriculture, forestry, fishing): 254 controls, 43 ALS: control(9.50%), ALS at 23.10%, OR=2.69 (1.40-5.16). 3. Secondary industry(ironwork, construction, chemical handling, traffic, transportation):control, 244 subjects, ALS, 53 subjects: Control at 12.7%, ALS at 26.90%, OR=2.81(1.45-5.46) 4.Tertiaryindustries(service, office, business): control, 214 subjects, ALS, 83 subjects: Control at 31.7%, ALS at 21.30%, OR=0.54(0.30-0.98).
	Provinciali L, 1990, Italy	Case control	1. 77 patients (57 males, 20 females, mean age=59±8) from 89 consecutive patients from a clinic during 1979-1987. 2. Controls (80) with various types of neuron diseases (including infectious diseases)were matched by age, sex, life-style(alcohol drinking, smoking), education, origin, cultural background from in patients over same period	No race information.	Fracture--strenuous work- hard work 1. Hard labor(exposed/non-exposed): Pa=54/23, CTL=39/41, OR=2.47(1.74-3.13). 2. trauma(fractures/no): Pa=19/58, CTL=12/68, OR=1.86(1.06-2.66)
	Strickland D, 1996, USA, Minnesota	Case control	1. Selected from Mayo clinic. 25 cases were selected. Patients with severe mobility and communication limitations were excluded. 2. Clinic control was selected from same clinic with neuromuscular disease, gender and age matched 2:1.	1. 25 cases, 25 clinic controls, and 25 community controls 2. Males/females proportion was 52%. Average ages was 56.2y in cases, 55.2 in clinic controls, and 56.1 in community controls. Age range is 29-81	Trauma strenuous work sports 1. Trauma (head, neck, back severe injury): 40% (10 subjects) in cases, 20% (5 subjects)in clinic controls, 16% (4 subjects) in community controls. OR=5.3(1.7-17.0). Cases who answered yes were younger than controls (35Y, 39Y and 47Y respectively). 2. Strenuous work (Sweating): OR=1.6(1.1-2.4). OR=3.1(1.04-9.30)

<table>
<tr><td></td><td></td><td></td><td>3. Community control: First 5 digital phone number matched, and gender and age matched. 2:1
4. They were mailed a set of interview forms to help them to prepare and recall question.
5. They were asked to come to the clinic to be interviewed by a nurse interviewer</td><td></td><td>3. for high school organized sports; OR=3.4(1.1-9.9) for organized sports at childhood or adolescence</td></tr>
<tr><td></td><td>*Buckley J, 1983, England</td><td>Registration</td><td>Not mention</td><td>cases=356 + 346 + 335, from mortality data</td><td>Strenuous work--Construction
1.The AMR in construction workers was increased by 36%.
2. Skilled (manual or non-manual) workers have higher than expected AMR.</td></tr>
<tr><td></td><td>*Imaizumi Y, 1986, Japan</td><td>Registration</td><td>Surveillance over a 10 year period(1969-1978)</td><td>Totally 3437 ALS cases were reported from surveillance data.</td><td>Strenuous work-blue collar, white collar
Occupations: In contrast to the conclusion from Italy or Brazil, the mortality rate in blue collar workers was not higher (obs=522/exp=520), but significantly excess deaths were observed in white collar workers (obs=563/exp=472).</td></tr>
<tr><td></td><td>*Werneck LC, 2007, Brasil</td><td>Registration</td><td>1. all the cases (283) diagnosed as motor neuron disorders attended a clinic between 1977 and 2004, 32 were excluded.</td><td>Sporadic ALS: spinal onset, 144 males, 76 females; bulbar onset, 9 males, 15 females.
Familiar ALS: 4 males, 3 females.</td><td>Strenuous work
the workers involved industrial good production and providing service (cases=56, SMR=1.38) represent higher risk than expected.</td></tr>
<tr><td></td><td>*Gunnarsson LG, 1991, Sweden</td><td>Cohort Study</td><td>1. 4 million Sweden residence were born 1896-1940. 1960 census.
1. 1970 -1983 all death cases were identified</td><td>1. 1961 ALS cases, 1130 males, 831 females. 1067 males with occupations, 308 females with occupations.
2. 2245 controls were randomly selected. around 250 from every 5 consecutive year birth cohort.1080 males, 1165 females. 1005 males and 429 females have occupations.</td><td>Strenuous workers:
1. Concrete/building workers 52, 1.4(0.9-2.3)
2. Unskilled workers, 37, 1.7(0.9-3.0)</td></tr>
<tr><td></td><td>*Park RM, 2005, USA</td><td>Cohort Study</td><td>1. Death certificate information for all deaths from 22 participating states in the years 1992–1998 was obtained using the National Occupational Mortality Surveillance System
2. Count Underlying and contributing causes of death
3. Controls were all decedents with no mention of neurologic disease, degenerative or otherwise, and excluding: accidental causes, malignant neoplasms of the brain (ICD 191), other senile and presenile organic psychotic conditions (ICD 290), diseases of the nervous system and sense organs (ICD 320–389), and finally, neoplasms of the lymphatic and hematopoietic tissues (ICD 200–208), due to suspect associations with solvents or electromagnetic fields (EMFs).</td><td><table><tr><th></th><th>Motor neuron disease related death</th><th>Total death</th></tr><tr><td>White men</td><td>3,851</td><td>1,479,921</td></tr><tr><td>White women</td><td>2,152</td><td>803,110</td></tr><tr><td>Non-white men</td><td>203</td><td>203,862</td></tr><tr><td>Non-white women</td><td>141</td><td>127,453</td></tr><tr><td>Total</td><td>6,347</td><td>2,614,346</td></tr></table></td><td>Strenuous-blue collar-with vibration-military
1. Bus drivers: MOR=1.61(1.09-2.28) P=0.011
2. Dental assistants: MOR=3.18(1.36-6.63) P=0.004
3. Food counter and fountain and related: MOR=3.38(1.04-7.96) P=0.016
4.Graders and sorters, excluding agriculture: MOR=2.20(1.00-4.13) p=0.028
5. Hairdressers and cosmetologists: MOR=1.38(1.00-1.87) p=0.046
7.Precision textile, machine workers MOR=1.94(1.30-2.78) p<0.001
9. Veterinarians: MOR=2.68(1.13-5.33) p=0.011
Note: MOR=mortality odds ratio</td></tr>
<tr><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Lower education</td><td>Gallo, 2009, 10</td><td>Cohort Study</td><td>1. 517,890 eligible healthy subjects aged 35 to 70 years were recruited from the general population residing in a given geographical area in a period</td><td>1. 116 cases, 517,890 population size
2. Mean age: Cases (M, 59.2; F, 60.1); Cohort(M, 52, F, 52.6)</td><td>Lower Education
1. No or primary education (%): Cases (yes/no)=52/64 (44.5), cohort N=153,319 (30.3), p=0.007</td></tr>
</table>

	European countries		ranging from 1991 to 2001, in 23 centers across 10 European countries. 2.At recruitment, information on lifestyle and dietary habits was collected through standardized questionnaires. 3.follow-up time between December 2001 and December 2005, generating a total of 4,591,325 person-years. For the rest of the analysis, 12,411 subjects (2.4% of the entire cohort) with missing information on smoking status at recruitment were excluded (including 2 ALS cases).	3. Follow up for 10 years at average 4. Data for race, onset, type of ALS were not available.	2. Higher education(%): Cases(y/n)=58/58 (50.0), cohort (y/n)=334760/153,319. 3. Marital difference: Less single, more Widowed in ALS patinets than controls 4. Less employment at the entry for both males and females: Employment at the entry: ALS: Male 20 (58.8%), females 19 (30.7%), cohort male 97,341 (73.7%), female 207,862 (64.3%); Total ALS 39 (40.6%) total cohort 305,203 (67.0%) P=0.001
	Kamel F, 2002, USA	Case control	Has been described in other papers	No difference between two groups with regard to the age, sex ratio, education level	lower education inactivity 2. Lower education(yes/no): 38/71, 56/200, OR=2.0(1.2-3.3)
	Sutedja NA, 2007, Netherlands	Case control	1. A total of 364 of 482 ALS (76%) and 392 of 498 control (79%) returned questionnaires 2. Blind to analysts only	1. 364 cases, 392 controls. 2. Average age, cases 60.2y, Control 60.0Y 3. Onset age: 58y 4. Bulbar, 96; spinal, 252; 5. Passible70,suspected 30; definite/probable 241.	Strenuous work, lower education, Education--Occupation 1. Low level of education (elementary school) (OR = 2.2 (1.2 -3.8), p < 0.01). 2. Among women whose main occupation was classified as crafts and related trades workers (OR = 8.4(1.0-70.1), p = 0.05).
BMI	Beghi E, 2010, Italy, UK, and Ireland	Case control	1. P: have to be definite, probable, or possible ALS cases, did mention when the study was done 2. Age (±2.5 y) and sex matched for control for each patient, selected by general practitioners for the patients 3. Report response rate is more than 90%, no further data	1. Cases/controls=61/112. P, 27 F, 34 M; C, 46 F, 66 M, not exactly matched. 2. Age, marital, education, no difference between P and C.	Trauma: head, sport, strenuous workers, professional sports 1. All trauma (yes/no), no difference: P=29/32, C=59/53 2. Head trauma (yes/no): no difference: P, 4/57; C, 11/101 3. Repeated trauma (yes/no): P, 21/40; C, 27/85, OR=1.65(0.97-2.33) 4. Work intensity (Yes/no, P<0.05): Strenuous/all, P=8/53, C=4/108; 5. Sport intensity (yes/no, P>0.05): strenuous/all others, p=8/53, C=19/93 6. Professional sport(yes/no, p=0.04):P=3/58, C=0/112 BMI BMI. cases: 25.3, SD 4.5; controls: 26.1, SD 4.1 Details were not provided in this paper
	(49,176,219)(32,49,66)(32,49,66)Okamoto K, 2009, Japan	Case control	1. Of the 274 ALS patients, 214 (75.3%) eligible cases were enrolled, 183 of whom completed the entire questionnaire. 2.Two community controls that matched to each case for age (G2 years) and gender. 3.Among a total of 732 eligible controls contacted, 550 (75.2%) were enrolled in this study and 430 completed the entire questionnaire.	1. from 2000 to 2005 2. Cases (n 153) Controls (n 306),60.3% men for cases and controls 3. Mean age (G SD) 63.7±9.2 for cases 63.4±10.6 controls	BMI and Vigorous activities 1.BMI : 22.2±0.2 for cases, 23.3±0.3 for control P<0.05 2. Vigorous activity: 11.6% for cases 6.2% for control OR=2.0 (1.0–4.0).
	Sutedja ,J 2011, Hollands	Case control	1. Between 1 July 2004 and 1 July 2009, patients diagnosed as having sporadic ALS at the University Medical Centre Utrecht. 2. Controls were selected by patients (not spouse, relative, within 5 y, same sex), and also randomly selected by GP using same criteria.	1. Median age, range: for cases, 60(24-82); for controls, 59(29-89). 2. Female, N(%): For case, 145(43), for controls, 246(46). 3. Bulbar onset: 86(27%) 4. The response rate of the participants in the questionnaire study was 80%	BMI 1. Compared with controls, fewer patients used cholesterol-lowering agents (OR=0.6-0.9, p=0.008) or were overweight (OR=0.7, 95% CI 0.5 to 1.0, p=0.02); 2. Moreover, patients had a lower BMI (ALS BMI=25±3.5, control=26±3.6, OR=0.9, 95% CI 0.9 to 1.0, p=0.001). 3. TC and LDL were significantly lower, and HDL was significantly higher in

			3. 334 patients and 538 controls were included. 4. In the blood sample study, 303 patients and 2100 controls (from other sources) were included.		ALS patients. 4. The LDL/HDL ratio was significantly lower in patients with ALS (in women, OR=0.4, 95% CI 0.3 to 0.6, p<0.001; in men, OR=0.5, 95% CI 0.4 to 0.6, p<0.001).
	Scarmeas N, 2002, USA	Case control	The 431 subjects were consecutive patients seen between 1992 and 2000 in the practice of L.P.R	279 patients with motor neuron diseases and 152 with other neurologic diseases	Sports For varsity athletics (no/yes): Cases=144/88; control=89/32; OR=1.70(1.04-2.76). BMI-slimness subjects with motor neuron diseases were more likely than controls to report they had always been slim or they had been varsity athletes. For slimness(no/yes): Cases=79/160; control=61/56; OR=2.21(1.40-3.47)
	O'Reilly EJ, 2013,USA	cohort	The end of follow-up was June 2008 for the NHS, January 2008 for the HPFS, December 2006 for the CPS-II Nutrition, December 2007 for the MEC, and December 2008 for the NIH-AARP.	1153 participants with ALS in over 562,942 males and 537,968 females. Following exclusions of individuals with missing or extreme BMI, there remained 1124 participants with ALS among 552,455 males and 520,059 females.	Cohort-specific c Cox proportional hazards models were used to estimate rates that were then pooled with random effects models. Results showed that lower BMI at baseline was associated with ALS; for each 5-unit increase in BMI, ALS rates were 21% lower (95% CI 14% – 27%). Compared to individuals with healthy BMI, ALS rates were significantly lower among the overweight (RR = 0.76 (95% CI 0.62–0.93)) and obese (RR =0.73 (95% CI 0.55–0.96)). Among never smokers the association persisted: RR 0.75 (95% CI 0.65–0.85) for each 5-unit increase. Excluding the first seven years of follow-up, the associations were materially unchanged suggesting that weight loss from undiagnosed disease does not fully explain the findings. Overall, 75% of males and females had a healthy BMI at age 18/21 years, 15% of males and 8% of females were overweight or obese;
	*Okamoto K, 2009, Japan	Case control	1. Of the 274 ALS patients, 214 (75.3%) eligible cases were enrolled, 183 of whom completed the entire questionnaire. 2.Two community controls that matched to each case for age (G2 years) and gender. 3. Among a total of 732 eligible controls contacted, 550 (75.2%) were enrolled in this study and 430 completed the entire questionnaire.	1. From 2000 to 2005 2. Cases (n 153), Controls (n 306), 60.3% men for cases and controls. 3. Mean age (G SD) 63.7±9.2 for cases 63.4±10.6 controls.	Fracture-sports: 1. Fracture: 19.6% for cases, 15.1% for control, OR=1.3 (0.9–2.1). 2. Vigour activity: 11.6% for cases, 6.2% for control OR=2.0 (1.0–4.0)

Chapter 7. General conclusions, study limitations, and recommendations

7.1. General conclusions

In this study, we reviewed the genetic and environmental risk factors associated with the development of ALS by employing systematic review and meta-analysis methods. We newly identified one genetic risk factor (CAG intermediate repeat expansion in *ATXN2*), two physical risk factors (experience of injury and electric shock), three environmental factors (exposure to pesticides, solvents, and lead), and one life style risk factor (former smoking) as being associated with ALS onset. Specifically, we systematically reviewed the associations between ALS and the four most important risk factors separately (i.e., CAG intermediate repeat expansion in ataxin-2, exposure to lead, exposure to pesticides and experience of trauma) in depth in four separate manuscripts. We found that the maximum attributable risk to ALS due to exposure to each risk factor was about 5% of all SALS cases. We also identified scientific evidence demonstrating that a few factors (intake of vitamin E, suboptimal BMI, and participating in physical activity) might play a protective role in the development of ALS. Monogenic mutations in *SOD1* and *C9ORF72* could explain about 25% and 35% of total FALS cases (FALS is composed of 5-10% of total ALS), respectively. The monogenic mutations identified so far in about two dozen genes could explain about 10% of all ALS cases (3). All environmental risk factors together play roughly equally important roles in the aetiology of ALS (17,18). Therefore, more genetic and environmental risk factors for ALS will likely be identified, since the identified risk factors are not sufficient to explain the full burden of ALS. Consistent with previous hypothesis, this project further demonstrated that many genetic and environmental risk factors contributed to the occurrence of ALS. Future studies are required to verify the findings in this project, and to explore additional genetic and environmental causes of ALS.

7.2. Limitations

7.2.1. Data source limitations: The diagnosis of ALS remains a challenge for neurologists, even though ALS disease was recognised more than 150 years ago (172). The

real challenge for the diagnosis of ALS is that the causes for most of ALS cases are still unknown, in addition to the lack of an objective laboratory test to be used in clinical diagnosis. ALS cases are diagnosed into three categories (definite, probable, or possible), according to disease differential diagnosis based primarily on clinical manifestations (38). In these situations, the ALS cases recruited into the studies included in this project may demonstrate different ratios of the three types of ALS cases among included studies (especially for 'possible' ALS cases). Some studies may include fALS cases, which are mainly associated with monogenic mutations. In addition, different countries adopt different diagnostic criteria for the diagnosis of ALS, especially prior to the introduction of EI Escorial criteria (38). Finally, ALS diagnostic criteria continue to evolve, and new ALS variants continue to be identified (39), leading to temporal variation in diagnostic criteria for ALS.

7.2.2. Limitations of meta-analyses: The readers should bear in mind that the association between ALS and a risk factor obtained from a meta-analysis remains an association only, not causation, regardless of how many selected studies were included. Secondly, although the combination of multiple studies was able to significantly increase the sample size and statistical power of the meta-analyses, the results might not be always significantly consistent with those from a large observational study, because the meta-analysis was designed to calculate a combined estimate based on the estimates from each of the included studies (428,429). Thirdly, the meta-analysis itself was unable to uncover publication bias in some instances, although the statistics for heterogeneity and the funnel plots among included studies were provided from the software RefMan5.1. Journals tend to accept positive results for publications, or publish results consistent with leading articles. In this instance, meta-analysis of the articles with same type of publication bias would lead to no publication bias conclusion. Fourthly, the meta-analysis was designed better for pooling results from various intervention studies conducted according to similar study design, test medication, type of patients and corresponding controls (429). However, observational study designs vary among the studies included in this project. The overall risk estimates from articles with lower quality was slightly inflated, compared to the estimate from articles with higher quality (see the lead manuscript). In this case, we have to assume that study design for every included study is identical, although this assumption does not hold strictly true for observational studies of risk

factors for ALS. Finally, although we have attempted to evaluate the quality of included studies, meta-analysis itself does not give a publication quality-weighted estimate.

7.2.3. Limitation of analysis process: The meta-analyses were conducted mainly using prevalence data. Because of limitations imposed by the small number of included studies in this thesis, we are unable to do further subgroup and sensitivity analysis in most meta-analyses, or run meta-regressions. Thus, the estimates based on meta-analysis in this project were not adjusted for potential confounding factors in most circumstances. In addition, we are unable to quantify the interaction between different risk factors identified in this project in our analyses.

7.2.4. Limitation of the estimates of attributable risk: To help general public, and medical professionals to understand the contribution of each risk factor to the aetiology of sporadic ALS, we have introduced the calculation of attributable risk in this thesis according to the Breslow and Day's statistical method (338). To do so, we assume that the studied risk factor could cause ALS, the populations of cases and controls in included articles are representative to general populations, and the excess exposure to this risk factor is responsible for the cases who reported that they had been exposed to this risk factor. Apparently, these are not true. These assumptions will lead to extremely overestimation of the associated risk due to exposure to a given risk factor. To make an approximate estimate, we use 'maximum' to further define the attributable risk. However, if we have the risk estimates from multiple level exposures, we would be able to adjust this risk estimate closer to the 'real' risk, if it is a true risk factor, just like Wartenberg's method (430). In addition, we could not estimate the combined attributable risk by examining the relative contribution of each risk factor, and their interactions. We have also compared the estimations of maximum attributable risks for the identified risk factors with other method (431), we felt that method used was the most reasonable method based on the available data.

7.3. Potential mechanisms: We have discussed the possible link between risk factors (exposure to heavy metals, exposure to pesticides, previous experience of injury, and genetic factors) and ALS in more detail in four separate manuscripts. The common pathological change was cellular inclusions of aggregated unfolded proteins (85). With the increase of

aggregated proteins, the cellular function of affected motor neurons was diminished slowly, until completely loss due to the apoptosis of affected motor neurons. The process of protein aggregation is initiated by the accumulation of unfolded proteins (363), since the production of unfolded protein exceeds the digestion capacity in ALS affected motor neurons. We have proposed that the presence of heavy metals in motor neurons leads to the excessive production of unfolded metal binding proteins in motor neurons (see manuscript on for lead exposure). However, pesticides might cause protein accumulation in motor neurons through the production of oxidative free radicals (405). Currently, it is difficult to link other risk factors such as brain injury to unfolded protein accumulation in motor neurons. In the future, the elucidation of mechanisms involved in unfolded protein accumulation in motor neurons after exposure to various related risk factors for ALS warrants further investigation in mechanistic animal studies.

7.4. The direction of future epidemiological studies: The risk factors identified in this project require further study in better designed observational studies in human populations, notably in cohort studies. However, a classical cohort study design is impracticable for rare diseases like ALS. ALS disease or death registry datasets for ALS at the national or provincial level would be helpful in identifying risk factors for ALS. This type of data has been available in some countries, such as Sweden, for a long time. Recently, there have been calls for the establishment of a registry for ALS in Canada (21). Some risk factors (such as occupation, trauma, educational attainment, residence, and smoking) have been proposed for inclusion in such a registry. However, the disadvantage of the disease specific registry is the lack of a corresponding control population needed for identification of risk factors. An alternative database for identifying risk factors for ALS is the Canadian national mortality database (432), like the study in America (150). In this instance, the selection of a control population is not an issue, and any investigator bias associated with the documentation of risk factors will be comparable between cases and controls, since all information on these risk factors was not obtained for a specific disease alone.

7.5. Recommendation to Public health: The risk factors associated with ALS identified in this project are not specific for ALS, but may also be detrimental to human health in other organs or tissues. The damage to other organs by these risk factors may be far

more important than the triggering of ALS. Therefore, avoidance of these risk factors does not only reduce the probability of developing ALS, but also would serve to promote population health in general. Thus, taking the necessary protective measures for workers with occupational exposure to pesticides (use pesticides as an example) does not only reduce the risk of developing ALS, but could also reduce the risk of other illnesses related to the toxicity of pesticides. On the other hand, we have identified some factors that play a protective role in the development of ALS. We therefore propose to take following measures in order to reduce the incidence of ALS.

7.5.1. Genomic sequencing: If possible, when whole genome sequencing for each individual becomes practical, ALS genes should be identified and communicated to the individual involved. If an individual is a monogenic mutant carrier of ALS, a healthy life style, including participating in physical activity and maintaining a reasonable body weight, should be followed. If he/she is a gene risk variant carrier of ALS, exposure to potential related environmental risk factors should be avoided. For example, if carrying a PON1 variant, the carriers should avoid an occupation involving exposure to pesticides.

7.5.2. Avoidance of exposure to potential environmental risk factors: Measures should be taken to reduce the amount and duration of exposure to pesticides, heavy metals, and organic solvents, and to reduce the chance of being injured. These activities do not only help to reduce the risk of having ALS, but also help to reduce the risk of developing other chronic diseases.

7.5.3. Adoption of a healthy life style: Participating in adequate and appropriate physical activities, consuming a healthy diet, smoking cessation, and taking nutrients (such as vitamin E) would help to reduce the population risk of developing ALS.

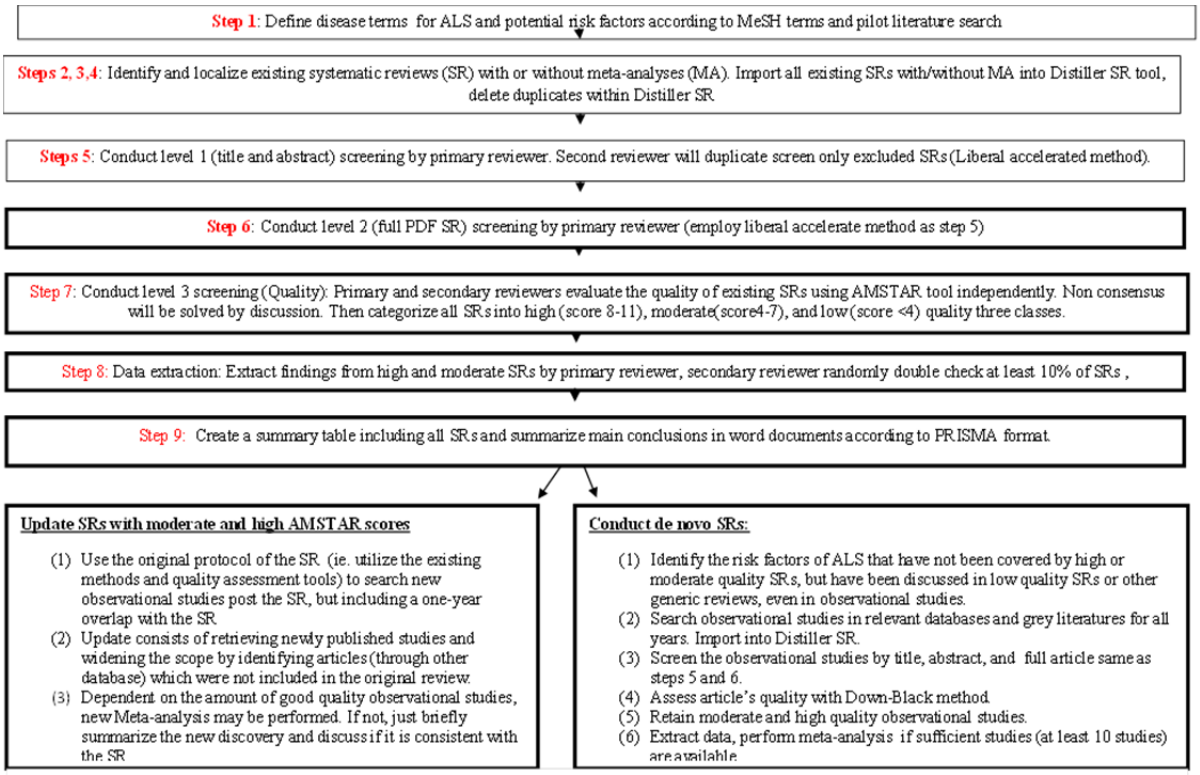
7.6. Intake of BMAA---A potential risk factor is drawing attention in literature: The incidence of ALS-PDC (ALS-Parkinson-Dementia-Complex) syndrome in Guam and surrounding Pacific islands was found to be 50-100 fold greater than the average incidence of ALS worldwide from the 1940s through to 1960s, but declined to similar level as seen in other regions after 1980, before its etiological factor was proven (433). The most

likely, but unproven, hypothesis for the cause of ALS-PDC in Guam and surrounding islands is the intake of the food toxicant Beta-N-methylamino-l-alanine (BMAA) (260-262). This hypothesis has re-emerged to explain the differences in ALS incidence in different regions (256,265,265,266). BMAA has been shown to induce ER stress (434), a critical event in the formation of unfolded protein accumulation. Despite the return of the BMAA hypothesis, a causal association still has not been established in human population-based studies. However, at present I personally tend to believe that BMAA is a risk factor for sALS. The disease term ALS-PDC was excluded from our ALS disease selection term, since we were not sure whether or not the ALS in Chamorro people was same as the sALS in other regions worldwide, and also because we believed that the environmental risk factor(s) associated with ALS-PDC was special to Guam and surrounding areas when we began with this project. Since the present study was designed to synthesize data from observational epidemiological studies, all studies related to BMAA would be excluded even if we included the terms of ALS-PDC because there is no relevant case-control or cohort study available for the ALS-PDC syndrome.

Regardless of whether or not this food toxicant theory is true, the story of temporary ALS epidemic in Guam and surrounding Pacific islands unequivocally indicates that toxic environmental factors are associated with ALS. An outbreak of SALS could occur in the presence of an appropriate environmental risk factor. Similar temporal fluctuations of ALS incidence, albeit to a much lower degree, have also been observed in many other areas worldwide (56,92,435,436), further underscoring the importance of environmental factors in the pathogenesis of SALS.

Appendix A: Method

A.1. General strategy for article search related to ALS



References:

1. Moher D. & Tsertvadze A. (2006). *The Lancet, 367:* 881-882.
2. Shea BJ, Grimshaw JM, Wells GA, Boers M, Andersson N, Hamel C, Porter AC, Tugwell P, Moher D, Bouter LM. BMC Med Res Methodol. 2007 Feb 15;7:10
3. White CM., Ip S., McPheeters M., et al. Using existing systematic reviews to replace de novo processes in conducting Comparative Effectiveness Reviews. In: Agency for Healthcare Research and Quality. Methods Guide for Comparative Effectiveness Reviews [posted September 2009]. Rockville, MD. Available at:http://effectivehealthcare.ahrq.gov/healthinfo.cfm?infotype=rr&ProcessID=60.
4. Whitlock EP., Lin JS., Chou R., et al. (2008).. *Annals of Internal Medicine, 148:* 776-782.
5. Downs SH, Black N. J Epidemiol Community Health 1998 Jun;52(6):377-384.
6. Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gotzsche PC, Ioannidis JP, et al. J Clin Epidemiol 2009 Oct;62(10):e1-34.

A.2. Searched databases and dates: A literature search strategy was designed based on Medline search using a set of terms for ALS disease, genetic and environmental risk factors. The search strategy was refined based on preliminary search results. Once optimized, the strategy was used to search relevant articles from other databases. Additional databases searched were Pubmed, EMBASE, Toxline/toxnet, Ageline, proquest, Psycinfo, Hugenet. The articles were searched for all the years through to March 18, 2013. Similar articles were tracked from Pubmed using disease terms only at least once a week until the thesis submission. Grey literatures were searched through Google scholar using the same search criteria as above. Some articles were identified from the reference lists of included articles, obtained from the requests to RACER via interlibrary loan, or obtained by contacting the corresponding author directly.

Existing systematic reviews and meta-analyses were searched first, then existing observational studies were searched. The search protocol that was used is described below:

A.2.1. Search strategy for systematic review, meta-analysis and observational studies using lead as an example of the risk factors: This is a common search strategy for systematic reviews for exposure to lead and disease ALS from databases Medline, Psycinfo, and Embase. For the search with the other databases, terms may need to be adjusted to fit to the database. To search other risk factors, just change step 15. For search observational studies related exposure to lead, then just skip step 1 to step 8.

1. Meta-Analysis/
2. (meta anal* or metaanal*).ab,sh,ti.
3. 1 or 2
4. (methodol* or systematic* or quantativ*).ab,sh,ti.
5. 3 and 4
6. (((methodol* or systematic* or quantativ) adj review*) or overview* or survey*).ab,sh,ti.
7. review.pt,sh.
8. 6 and 7
9. 5 or 8

10. amyotrophic lateral sclerosis/ or motor neuron disease/ or Lou Gehrig's disease/

11. (als or mnd or motor neuron* disease or motor neuropath*).mp.

12. 10 or 11

13. exp risk/

14. etiology.fs.

15. (lead, heavy metals, welding, soldering).mp.

16. 13 or 14 or 15 or 16

17. 12 and 17

18. limit 18 to humans

19. 9 and 19 20. limit 20 to (english or French)

A.3. Record screening, data extraction from searched systematic review and meta-analyses

A3.1. Screening: All the relevant articles were collected into Ref-manager or RefWorks, and further migrated into DistillerSR for screening. Additional manually identified articles were also added into this database. The duplicate records were removed using Ref-manager, or in DistillerSR. The retained articles were screened by reading title and abstract against including/excluding criteria (disease terms, study types, human study, article types), and then all the relevant and not-sure articles were retained. The full PDF articles were found and uploaded to DistillerSR database, and further examined for relevance and appropriateness with a priori inclusion/exclusion criteria (disease terms, risk factors, and systematic review and meta-analysis).

A3.1.1 .General inclusion and exclusion criteria:

1. Study must be related to ALS or MND.

2. Study must be systematic review or/meta-analysis.

3. Study must be designed to investigate at least one genetic or environmental risk factor.

4. Letters, commentaries, generic reviews were excluded. Conference abstracts were also excluded if full articles were not able to find.

5. For a SR/MA, the quality score must be >=4.

A.3.2. Evaluation and data extraction: Selected systematic reviews and meta-analyses were evaluated using AMSTAR scale with 11 questions (1 score/question). The scores were totaled and categorized into three categories (AMSTAR score of 0-3: low; AMSTAR score of 4-7: moderate; and AMSTAR score of 8-11: high) (22). The systematic reviews or meta-analyses with moderate or high quality will be retained, and main results and conclusions and other key information will be extracted in DistillerSR with a predesigned form. The score systems were listed below:

A.3.2.1. How to score a SR article based on AMSTAR scores:

1. Was an 'a priori' design provided?

2. Was there duplicate study selection and data extraction?

3. Was a comprehensive literature search performed?

4. Was the status of publication (i.e. grey literature) used as an inclusion criterion?

5. Was a list of studies (included and excluded) provided?

6. Were the characteristics of the included studies provided?

7. Was the scientific quality of the included studies assessed and documented?

8. Was the scientific quality of the included studies used appropriately in formulating conclusions?

9. Were the methods used to combine the findings of studies appropriate?

10. Was the likelihood of publication bias assessed?

11. Was the conflict of interest included?

A.3. 3 Summary of searched results for SA/MA articles:

1. Records found from database search for SR/MA (581 articles found)
2. Screen titles, abstracts against criteria (English articles, human study, with disease terms, risk factors, not observational studies, commentary). 129 articles were included.

3. Screen full articles in DistillerSR against inclusion criteria (systematic review or meta-analysis for ALS). 6 articles were included.
4. Four SR and two MA articles were evaluated with AMSTAR (22).
5. Six additional systematic reviews and meta-analyses were identified via PUBMED tracking (data not extracted since, after step 4, we decided to obtain all relevant observational studies to run our own systematic review and meta-analyses).

A.3.4. Summary of systematic reviews:

1. Two SR articles with moderate scores: One is for Chemicals: no association was identified; the other is for occupation: no association was identified.
2. Two SR articles with defects: One is for smoking: data synthesis process was not appropriate; the other is for electromagnetic field: did not synthesize data.
3. Two Meta-analyses for genetic factors with lower AMSTAR score: One is for PON1, no association; The other one is for VEGF, no association.
4. Additional six articles were found, but we did not score them, since we had decided to re-do all risk factors using systematic review and meta-analysis.

A.3.5. General Conclusion: No solid association has ever been identified in existing SR/MA articles. Two new meta-analyses about pesticides were published in 2013 after we decided to run our own SR/MA. The main conclusion is the same as we got from our own meta-analysis.

A.4. Record screening, data extraction from searched observational studies

A.4.1. Screening: All the relevant articles were collected into Ref-manager or RefWorks, and further migrated into DistillerSR for screening. Additional manually identified articles were also added into this DistillerSR database. The duplicate records were removed using Ref-manager, or in DistillerSR. The retained articles were screened by reading title and abstract against including/excluding criteria (disease terms, environmental risk factors, observational study including cohort, case-control, cross-sectional study), and then all the relevant and not-sure articles were retained. The full PDF articles were found and uploaded to DistillerSR database, and further examined for relevance and appropriateness with a

priori inclusion/exclusion criteria (disease terms, risk factors, observational study). This step was conducted by two reviewers.

A3.1.1 .General inclusion and exclusion criteria:

1. Study must be for ALS or MND.
2. Study must be an observational study including case-control, cohort, cross-sectional.
3. Study must be designed to investigate at least one genetic or environmental factor.
4. Letters, commentaries, reviews of all types were excluded. Conference abstracts were also excluded if full articles were not able to find.
5. For an observational study, sample size must be more than 30 (this filter was removed when dealt with important risk factors for individual manuscripts), and the controls must be matched by sex, and ages, and randomly selected.

A.4.2. Sort articles into different categories according to risk factors: The selected articles were sorted into multiple groups based on risk factors by designing an extra-column in extraction form for each risk factor in DistillerSR. In this case, we can have the DristillerSR sort the outputs based on risk factors.

A.4.3. Data extraction: Data (first author, country, published year, case ascertainment, case recruitment time/ location/ method of data collection, statistical method, main results, interest conflict) were extracted from the selected observational studies using predesigned form in DistillerSR.

A.4.4. Article assessment: Since the number of included observational studies is very limited, we did not use the article quality assessment to exclude articles. Instead, when need to run subgroup meta-analysis (low quality articles versus higher quality articles), the articles were assessed with modified downs and black criteria (282).

A.4.4.1. PHAC-Modified Downs and Black Study Quality Assessment Tool (Case-control Studies):

FACTOR	**SCORE**
External Validity	
1. *Were the subjects asked to participate in the study representative of the entire population from which they were recruited?* [1]Cases: "All eligible cases with outcome of interest over a defined period of time, all cases in a defined catchment area, all cases in a defined hospital or	1

clinic, group of hospitals, health maintenance organization, or an appropriate sample of those cases (eg. Random sample)" [1]Controls: Must be community controls. No points given for the use of hospital controls.	
2. *Were those subjects who were prepared to participate representative of the entire population from which they were recruited?* Participation rate for cases and controls of at least 70%	1
Subtotal	2
Internal Validity-Bias	
3. *Was an attempt made to blind those measuring the main outcomes of the intervention?* Exposure ascertainment was based on interviews blinded to health outcome status, mailed questionnaire, or other pre-existing or documented exposure information. Ex: interview without mention of blinding would get no points.	1
4. *If any of the results of the study were based on `data dredging`, was this made clear?* The study was designed to examine the reported association.	1
5. *In case-control studies, is the time period between the intervention and outcome the same for cases and controls?* Cases and controls were age matched and the exposure period examined was well-defined.	1
6. *Were the statistical tests used to assess the main outcomes appropriate?* The statistical techniques used were appropriate for the study design and sample size.	1
7. *Was compliance with the intervention reliable?* The effect of exposure misclassification was likely to bias the reported association towards the null. For example, exposure status based on pre-existing or documented exposure information (not retrospective case interviews).	1
8. *Were the main outcome measures used accurate (valid and reliable)?* Outcome measurement was clearly described and was virtually certain (histologically confirmed cancer cases).	1
Subtotal	6
Internal Validity-Exposure Measurement	
9. [a]*Were measures of exposure robust?* Exposure status was either documented or determined via biomarker (2); used small ecological measures, job titles, or was self-reported (1); was based on large ecological measure (0).	2
10. [a]*Was there a sufficient exposure gradient?* The degree of variability between categories of exposure frequency, duration, or intensity was high (2); medium (1); low or unknown (0).	2
11. [a]*Were measures of exposure specific?* Exposure measures were specific (2); based on broader, chemically related groups (1); based on broad groupings of diverse chemical and toxicological properties (0).	2
12. [a]*Were all critical exposure time windows measured and reported?* Exposure time windows were all (2); partially (1); or not at all defined, measured, and	2

reported (0).	
Subtotal	8
Internal Validity-Confounding	
13. *Were the cases and controls recruited from the same population?* Information on the source of study participants provided; controls representative of the study base from which cases are drawn.	1
14. *Were the cases and controls recruited over the same period of time?* The calendar period over which cases and controls were recruited was defined and similar.	1
15. *Was there adequate adjustment for confounding in the analyses from which the main findings were drawn?* The study collected data on potential confounders and assessed their effects in analysis: Included other covariates in addition to age and sex (2); age and sex only (1); none (0)	2
Subtotal	4
Total	20

[-1]Select components of the Newcastle-Ottawa Scale (NOS) were used to help clarify certain prompts within the Modified Downs and Blacks tool.
Reference: Wells GA., Shea B., O'Connell et al. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomized studies in meta-analyses. Retrieved June 29, 2011 from http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp.

A.4.5. Summary of searched observational studies

1. 5638 articles were screened with titles by against inclusion/exclusion criteria [English, human study, disease terms, risk factors, and observational epidemiological study (not review, commentary, or intervention study)].
2. 1071 articles were screened with abstracts against inclusion/exclusion criteria same as above.
3. 397 articles were screened with full PDF articles against inclusion/exclusion criteria same as above, plus with sufficient information about studied population, more than 30 cases, more than 30 controls, 5 or more cases in each cell of a contingency table, diagnostic criteria stated, data analysis methods indicated, risk factor information listed, clear description of the results.

A.4.6. Summary of included studies: Data were extracted from 88 articles (Note: many studies were related to multiple risk factors).

1. Studies of smoking: 24 articles
2. Studies of exposure to pesticides and involved in agricultural activities: 24 articles

3. Studies of trauma, and related topics: 36 articles
4. Studies of heavy metals: 16 articles
5. Studies of previous exposure to solvents: 11 articles
6. Studies of the ALS and other comorbidities: 14 articles
7. Studies of exposure to magnetic field: 22 articles
8. Studies of alcohol drinking: 9 articles
9. Studies of other risk factors for ALS: 70 articles

A.5. Genetic studies

A.5.1. Searched databases: ALSod and Pubmed. The information of monogenic mutation and meta-analyses of the relationships between ALS and different gene variants were extracted from ALSod. This information was summarized and added into literature review. Here we mainly focused on one genetic risk factor, ataxin-2.

A.5.1. Search strategy for ataxin-2

A.5.1.1. Search through medline, psychinfo, embase

1. amyotrophic lateral sclerosis.mp. [mp=ti, ab, sh, hw, tn, ot, dm, mf, dv, kw, tc, id, tm, nm, kf, px, rx, an, ui]
2. motor neuron disease.mp. [mp=ti, ab, sh, hw, tn, ot, dm, mf, dv, kw, tc, id, tm, nm, kf, px, rx, an, ui]
3. motoneurone disease.mp. [mp=ti, ab, sh, hw, tn, ot, dm, mf, dv, kw, tc, id, tm, nm, kf, px, rx, an, ui]
4. lou gehrig's disease.mp. [mp=ti, ab, sh, hw, tn, ot, dm, mf, dv, kw, tc, id, tm, nm, kf, px, rx, an, ui]
5. als.mp. [mp=ti, ab, sh, hw, tn, ot, dm, mf, dv, kw, tc, id, tm, nm, kf, px, rx, an, ui]
6. mnd.mp. [mp=ti, ab, sh, hw, tn, ot, dm, mf, dv, kw, tc, id, tm, nm, kf, px, rx, an, ui]
7. 1 or 2 or 3 or 4 or 5 or 6
8. ataxin-2.mp. [mp=ti, ab, sh, hw, tn, ot, dm, mf, dv, kw, tc, id, tm, nm, kf, px, rx, an, ui]
9. *ATXN2*.mp. [mp=ti, ab, sh, hw, tn, ot, dm, mf, dv, kw, tc, id, tm, nm, kf, px, rx, an, ui]
10. atx2.mp. [mp=ti, ab, sh, hw, tn, ot, dm, mf, dv, kw, tc, id, tm, nm, kf, px, rx, an, ui]
11. sca2.mp. [mp=ti, ab, sh, hw, tn, ot, dm, mf, dv, kw, tc, id, tm, nm, kf, px, rx, an, ui]
12. asl13.mp. [mp=ti, ab, sh, hw, tn, ot, dm, mf, dv, kw, tc, id, tm, nm, kf, px, rx, an, ui]
13. tnrc13.mp. [mp=ti, ab, sh, hw, tn, ot, dm, mf, dv, kw, tc, id, tm, nm, kf, px, rx, an, ui]

14. polyglutamine.mp. [mp=ti, ab, sh, hw, tn, ot, dm, mf, dv, kw, tc, id, tm, nm, kf, px, rx, an, ui]
15. polyq.mp. [mp=ti, ab, sh, hw, tn, ot, dm, mf, dv, kw, tc, id, tm, nm, kf, px, rx, an, ui]
16. cag repeat.mp. [mp=ti, ab, sh, hw, tn, ot, dm, mf, dv, kw, tc, id, tm, nm, kf, px, rx, an, ui]
17. 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16
18. 7 and 17
19. limit 18 to english language
20. limit 19 to human
21. limit 20 to yr="2010 -Current"

A.5.1.2. Search through Hugenet navigator

1. Select literature, enter disease term: amyotrophic lateral sclerosis
2. Filter with gene: *ATXN2*
3. 11 articles were exported to refwork.

***A.5.1.3. Search Pubmed*:** (ALS, MND, amyotrophic lateral sclerosis, motor neuron disease, motorneurone disease, or Lou Gehrig's disease) and (ataxin-2, *ATXN2*, *ATX2*, *SCA2, ASL13, TNRC13*, polyglutamine, or polyQ). 45 records.

A.5.2. Brief summary of search genetic studies

1. 104 reports were identified by ALSod database.
2. 22 monogenic mutation genes were identified (we quoted this information).
3. 12 gene variants were summarized with meta-analyses (We quoted this information).
4. We summarized ATAXN-2 with meta-analysis (articles were searched from pubmed and Embase, and 21 articles were identified. 13 articles were kept by screening with including criteria: disease terms, gene terms, study locations, data source, population characteristics, results, data-analysis).
5. The finding of this search was summarized in our first manuscript in this thesis.

Appendix B. Other findings via our meta-analyses based on included observational studies:

In addition to the findings listed above with the format of manuscripts, we also identified following associations between ALS and other environmental risk factors.

B.1. Previous exposure to solvents is associated with an increased risk of developing ALS:

Not a single article was identified that provided a definition for what solvent was. It was, therefore, assumed that all articles identified with solvent exposure have used the same definition for 'solvent'. Persons who have ever been exposed to solvents via occupations might also have also been exposed to other ALS related risk factors, such as heavy metals, organic phosphorus compounds, mechanical injuries, electric shock, etc. Therefore, even if we conclude a positive association between exposure to solvents and ALS, without subgroup analysis, or control for potential confounding risk factors, over-interpretation of the result should be avoided.

Ten of the included articles were selected into this analysis (141-152). Of these, one article was excluded because the prevalence data could not be located (144). Two cohort studies explored the associations between exposure to solvents and the risk of developing ALS. One article revealed a positive association (RR=1.16, 95%CI: 1.01-1.34) (150), but the other one showed no association (RR=1.05, 95%CI: 0.86-1.29) (149). Apparently, the latter one misclassified the occupation. The meta-analysis for the association between exposure to solvents and the risk for ALS was conducted with 7 case-control studies. The analysis revealed that the odds of exposure to solvents among ALS patients was higher than among controls, the OR was 1.43 (95%CI: 1.10-1.86) with random effects model, and 1.42 (95%CI: 1.13-1.80) with fixed effects model respectively, and with no significant publication bias identified for either model (random effects, $Tau^2 = 0.02$; $Chi^2 = 7.12$, df = 6 (P = 0.31); $I^2 = 16\%$); fixed effects, $Chi^2 = 7.12$, df = 6 (P = 0.31); $I^2 = 16\%$) (Figure A.1).

B.2. Life style

B.2.1. Alcohol drinking did not increase the risk for ALS, if it is not a protective factor. Ten case-control studies were selected for analysing the association between alcohol drinking and the risk of ALS (49,143,145,176,202,219,247,393,418). The synthesis of the prevalence data from 10 case-control studies with meta-analysis showed that there was no association between ever alcohol drinking and ALS with random effects model [OR= 0.95 (0.77-1.16)], but with a significant heterogeneity across included studies [$Tau^2 = 0.06$; $Chi^2 = 27.34$, df = 9 (P = 0.001); $I^2 = 67\%$](Figure A.2). Fixed effects model conferred a negative association [OR= 0.86 (0.78-0.96)] with significant heterogeneity across included studies [Fixed effects, $Chi^2 = 27.34$, df = 9 (P = 0.001); $I^2 = 67\%$)]) (Figure A.2). There is no publication bias identified according to funnel plot (data not shown).

B.2.2. Smoking-Existing SR/MA: An overall statistically not significant association between smoking and the risk of ALS was reported in a recent systematic review/meta-analysis (SR/MA), which synthesized 18 previous publications including 15 case-control and 5 cohort studies from Europe, USA and Japan (206). The risk of ALS in current smokers was 1.28 fold (RR, 95%CI: 0.97-1.68) of that in never-smokers, while the risk in ever smokers is 1.12 fold (RR, 95%CI: 0.98 - 1.27) of that in never smokers, not significantly different in both cases. However, there is a gender difference for the risk of ALS associated with smoking. The estimated RR (95% CI) of ALS for ever versus never smokers was 0.86 (0.71 - 1.03) in men, but a significant association (RR=1.66, 95%CI: 1.31-2.10) in women was found. Therefore the overall conclusion from this SR/MA is that smoking is not a strong risk factor for ALS. Here the real question is that why smoking is just associated with an increased risk of ALS among female smokers.

Comments and update*:* One defect of this existing SR/MA (206) is that the pooled analysis between cohort studies and case-control studies because the 'event' in these cohort studies is the disease ALS itself, whereas the event in those case-control studies is 'smoking'. If merge cohort and case control studies, the outcome in both types of studies must be the same, and the outcome event must be rare. Therefore, I reanalysed the selected case-control and cohort studies separately using meta-analysis approach, also include new publications

since then. Similar to the existing SR/MA analysis, the only significant difference with regard to the risk of ALS between ever smokers and never smokers was identified in female smokers from cohort studies [RR=1.34(1.17-1.55), random effects (Figure A.3) or RR=1.34(1.18-1.52), fixed effects].

B.2.3. Previous exposure to electromagnetic field via occupations may not be a risk factor for ALS, some positive associations may be confounded by chemicals, heavy metals, and electric shock: Nine cohort studies related to occupational exposure to electromagnetic fields have been selected. Although most studies showed excess death due to ALS among exposed groups, only 4 studies showed significant excess number of ALS cases. Since prevalence data could not be identified among most identified studies, synthesized conclusion based on meta-analysis could not be drawn. However, the intuitive impression is that exposure to electromagnetic field via occupations from these included cohort studies is associated with an increased risk of ALS.

Five case-control studies including one population based case-control study related to the risk for ALS among electric workers were identified. However, the prevalence data could not be identified from one of these studies, although it showed an association between some occupations related to exposure to electromagnetic field and increased risk for ALS, therefore this article is excluded. The meta-analysis from the remaining four case-control studies revealed that the risk for ALS was positively associated with previous exposure to electromagnetic field via occupation (random effects model, OR=2.02, 95%CI: 1.26-3.23, exposed electronic field via occupation among ALS versus among controls), with modest heterogeneity across included studies [$Tau^2 = 0.09$; $Chi^2 = 5.03$, df = 3 (P = 0.17); $I^2 = 40\%$] (data not shown). A similar association was also identified with meta-analysis recently (134). However, the occupations related to electromagnetic fields in these included studies could also be confounded by other potential ALS risk factors, such as heavy metals including lead and electric shock. However, these included studies do not allow us to perform subgroup analysis to control these confounding factors since the number of included studies is too small. Occupational welding has been associated with an increased ALS in a few studies, but not all. We have not identified enough studies to perform a meta-analysis for this.

B.2.4. Previous electric shock is associated with increased risk for ALS: Six case-control studies were selected to determine the association between previous electric shock and the following ALS diagnosis (128,145,327,415,416,419). Meta-analysis revealed a strong association for previous electronic shock and ALS with no detectable heterogeneity and publication bias across included studies [random effects, OR=3.27, 95%CI: 1.87-5.73, heterogeneity: $Tau^2 = 0.00$; $Chi^2 = 1.69$, df = 5 (P = 0.89); $I^2 = 0\%$. Fixed effects, OR= 3.43, 95%CI: 1.97-5.97, heterogeneity: $Chi^2 = 1.69$, df = 5 (P = 0.89); $I^2 = 0\%$] (Figure A.4). These results indicated that previous electric shock could be considered a risk factor for ALS.

Figures and legends

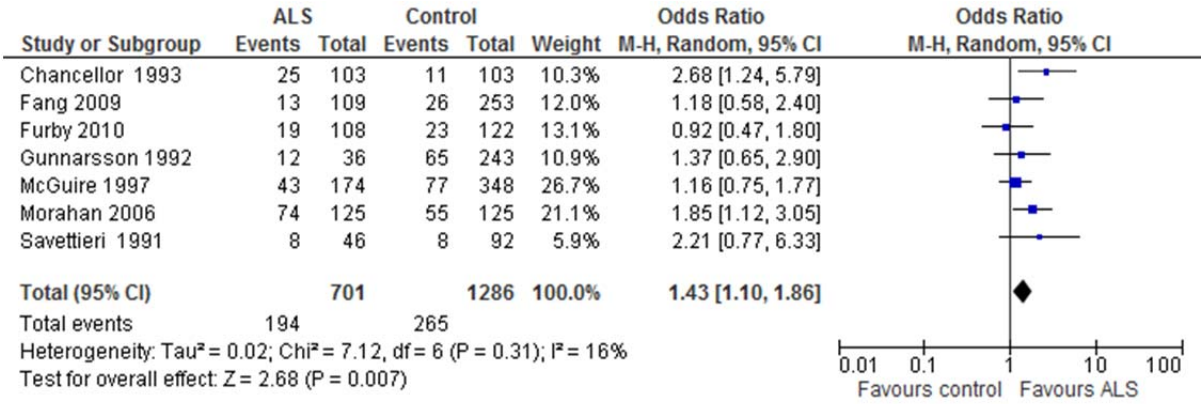


Figure B.1: Previous exposure to solvents is associated with ALS. The relationship between previous exposure to solvent (7 articles) and ALS was estimated using Revman 5.1 with random effects model. Significant publication bias was not noticed (data not shown).

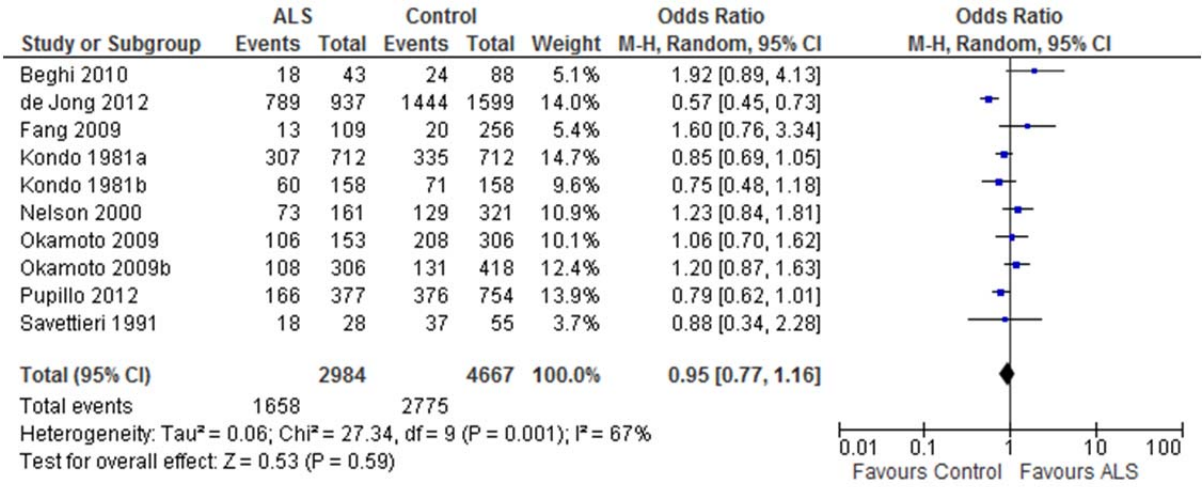


Figure B.2: Drinking alcohol is not associated with ALS. The relationship between drinking alcohol (10 articles) and ALS was estimated using Revman 5.1 with random effects model. Significant publication bias was not noticed (data not shown).

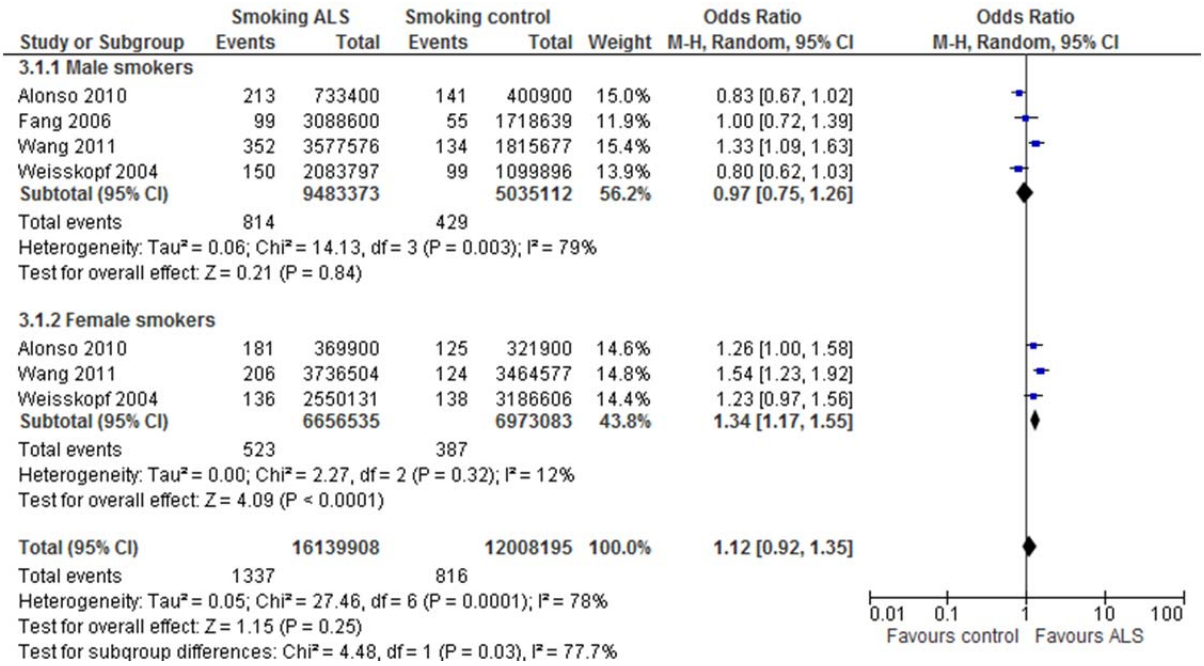


Figure B.3: Female smokers have higher risk of developing ALS. The relationship between female smokers (7 articles) and ALS was estimated using Revman 5.1 with random effects model. Significant publication bias was not noticed (data not shown).

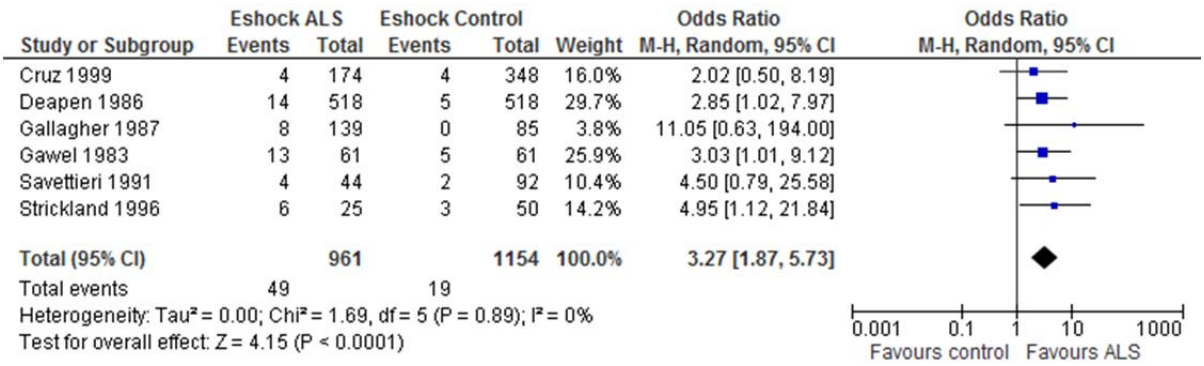


Figure B.4: Previous electric shock is associated with ALS. The relationship between electric shock (6 articles) and ALS was estimated using Revman 5.1 with random effect model. Significant publication bias has not been noticed (data not shown).

Bibliography

(1) Byrne S, Walsh C, Lynch C, Bede P, Elamin M, Kenna K, et al. Rate of familial amyotrophic lateral sclerosis: a systematic review and meta-analysis. 2011.

(2) Wijesekera LC, Leigh PN. Amyotrophic lateral sclerosis. Orphanet J Rare Dis 2009 Feb 3;4:3.

(3) Chio A, Calvo A, Mazzini L, Cantello R, Mora G, Moglia C, et al. Extensive genetics of ALS: a population-based study in Italy. Neurology 2012 Nov 6;79(19):1983-1989.

(4) Pfister T, Sekhon R, White M, Scott P, Munro S, Johnston M, et al. Familial amyotrophic lateral sclerosis in Alberta, Canada. Amyotroph Lateral Scler Frontotemporal Degener 2013 Jan 4.

(5) Robberecht W. Genetics of amyotrophic lateral sclerosis. J Neurol 2000 Dec;247 Suppl 6:VI/2-6.

(6) van Blitterswijk M, DeJesus-Hernandez M, Rademakers R. How do C9ORF72 repeat expansions cause amyotrophic lateral sclerosis and frontotemporal dementia: can we learn from other noncoding repeat expansion disorders? Curr Opin Neurol 2012 Dec;25(6):689-700.

(7) Smith BN, Newhouse S, Shatunov A, Vance C, Topp S, Johnson L, et al. The C9ORF72 expansion mutation is a common cause of ALS+/-FTD in Europe and has a single founder. Eur J Hum Genet 2012 Jun 13.

(8) Ratti A, Corrado L, Castellotti B, Del Bo R, Fogh I, Cereda C, et al. C9ORF72 repeat expansion in a large Italian ALS cohort: evidence of a founder effect. Neurobiol Aging 2012 Jul 4.

(9) Al-Chalabi A, Hardiman O. The epidemiology of ALS: a conspiracy of genes, environment and time. Nat Rev Neurol 2013 Nov;9(11):617-628.

(10) ALS ONLINE GENETICS DATABASE,World Federation of Neurology and European Network to Cure ALS . Jan. 2014;2014(01/13).

(11) Trojsi F, Monsurro MR, Tedeschi G. Exposure to environmental toxicants and pathogenesis of amyotrophic lateral sclerosis: state of the art and research perspectives. Int J Mol Sci 2013 Jul 24;14(8):15286-15311.

(12) Sutedja NA, Veldink JH, Fischer K, Kromhout H, Heederik D, Huisman MH, et al. Exposure to chemicals and metals and risk of amyotrophic lateral sclerosis: a systematic review. Amyotroph Lateral Scler 2009 Oct-Dec;10(5-6):302-309.

(13) Sutedja NA, Fischer K, Veldink JH, van der Heijden GJMG, Kromhout H, Heederik D, et al. What we truly know about occupation as a risk factor for ALS: a critical and systematic review. Amyotrophic lateral sclerosis : official publication of the World Federation of Neurology Research Group on Motor Neuron Diseases 2009 Oct-Dec;10(5-6):295-301.

(14) Chen S, Sayana P, Zhang X, Le W. Genetics of amyotrophic lateral sclerosis: an update. Mol Neurodegener 2013 Aug 13;8(1):28.

(15) Abel O, Powell JF, Andersen PM, Al-Chalabi A. ALSoD: A user-friendly online bioinformatics tool for amyotrophic lateral sclerosis genetics. Hum Mutat 2012 Jul 2.

(16) Sabatelli M, Conte A, Zollino M. Clinical and genetic heterogeneity of amyotrophic lateral sclerosis. Clin Genet 2013 May;83(5):408-416.

(17) Wingo TS, Cutler DJ, Yarab N, Kelly CM, Glass JD. The heritability of amyotrophic lateral sclerosis in a clinically ascertained United States research registry. PLoS One 2011;6(11):e27985.

(18) Al-Chalabi A, Fang F, Hanby MF, Leigh PN, Shaw CE, Ye W, et al. An estimate of amyotrophic lateral sclerosis heritability using twin data. Journal of neurology, neurosurgery, and psychiatry, December 01 2010;PMC2988617(12):1324-1326.

(19) Alonso A, Logroscino G, Hernan MA. Smoking and the risk of amyotrophic lateral sclerosis: a systematic review and meta-analysis. J Neurol Neurosurg Psychiatry 2010 Nov;81(11):1249-1252.

(20) Wolfson C, Kilborn S, Oskoui M, Genge A. Incidence and prevalence of amyotrophic lateral sclerosis in Canada: a systematic review of the literature. Neuroepidemiology 2009;33(2):79-88.

(21) Korngut L, Genge A, Johnston M, Benstead T, Bourque P, Briemberg H, et al. Establishing a Canadian registry of patients with amyotrophic lateral sclerosis. Can J Neurol Sci 2013 Jan;40(1):29-35.

(22) Shea BJ, Bouter LM, Peterson J, Boers M, Andersson N, Ortiz Z, et al. External validation of a measurement tool to assess systematic reviews (AMSTAR). PLoS One 2007 Dec 26;2(12):e1350.

(23) Turner MC, Wigle DT, Krewski D. Residential pesticides and childhood leukemia: a systematic review and meta-analysis. Cien Saude Colet 2011 Mar;16(3):1915-1931.

(24) Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D, et al. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. JAMA 2000 Apr 19;283(15):2008-2012.

(25) Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gotzsche PC, Ioannidis JP, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. J Clin Epidemiol 2009 Oct;62(10):e1-34.

(26) Sathasivam S. Motor neurone disease: clinical features, diagnosis, diagnostic pitfalls and prognostic markers. Singapore Med J 2010 May;51(5):367-72; quiz 373.

(27) Piaceri I, Del Mastio M, Tedde A, Bagnoli S, Latorraca S, Massaro F, et al. Clinical heterogeneity in Italian patients with amyotrophic lateral sclerosis. Clin Genet 2011 Jun 8.

(28) Talman P, Forbes A, Mathers S. Clinical phenotypes and natural progression for motor neuron disease: analysis from an Australian database. Amyotroph Lateral Scler 2009 Apr;10(2):79-84.

(29) Phukan J, Pender NP, Hardiman O. Cognitive impairment in amyotrophic lateral sclerosis. Lancet Neurol 2007 Nov;6(11):994-1003.

(30) Phukan J, Elamin M, Bede P, Jordan N, Gallagher L, Byrne S, et al. The syndrome of cognitive impairment in amyotrophic lateral sclerosis: a population-based study. J Neurol Neurosurg Psychiatry 2011 Aug 11.

(31) Gordon PH, Delgadillo D, Piquard A, Bruneteau G, Pradat PF, Salachas F, et al. The range and clinical impact of cognitive impairment in French patients with ALS: a cross-sectional study of neuropsychological test performance. Amyotroph Lateral Scler 2011 Sep;12(5):372-378.

(32) Cooper-Knock J, Hewitt C, Highley JR, Brockington A, Milano A, Man S, et al. Clinico-pathological features in amyotrophic lateral sclerosis with expansions in C9ORF72. Brain 2012 Mar;135(Pt 3):751-764.

(33) Byrne S, Elamin M, Bede P, Shatunov A, Walsh C, Corr B, et al. Cognitive and clinical characteristics of patients with amyotrophic lateral sclerosis carrying a C9orf72 repeat expansion: a population-based cohort study. Lancet Neurol 2012 Mar;11(3):232-240.

(34) Ludolph AC, Brettschneider J, Weishaupt JH. Amyotrophic lateral sclerosis. Curr Opin Neurol 2012 Oct;25(5):530-535.

(35) Seltman RE, Matthews BR. Frontotemporal lobar degeneration: epidemiology, pathology, diagnosis and management. CNS Drugs 2012 Oct 1;26(10):841-870.

(36) Testa D, Lovati R, Ferrarini M, Salmoiraghi F, Filippini G. Survival of 793 patients with amyotrophic lateral sclerosis diagnosed over a 28-year period. Amyotroph Lateral Scler Other Motor Neuron Disord 2004 Dec;5(4):208-212.

(37) Tosi L, Righetti C, Adami L, Zanette G. October 1942: a strange epidemic paralysis in Saval, Verona, Italy. Revision and diagnosis 50 years later of tri-ortho-cresyl phosphate poisoning. J Neurol Neurosurg Psychiatry 1994 Jul;57(7):810-813.

(38) Silani V, Messina S, Poletti B, Morelli C, Doretti A, Ticozzi N, et al. The diagnosis of Amyotrophic lateral sclerosis in 2010. Arch Ital Biol 2011 Mar;149(1):5-27.

(39) Kim WK, Liu X, Sandner J, Pasmantier M, Andrews J, Rowland LP, et al. Study of 962 patients indicates progressive muscular atrophy is a form of ALS. Neurology 2009 Nov 17;73(20):1686-1692.

(40) Chio A, Calvo A, Moglia C, Mazzini L, Mora G, PARALS study group. Phenotypic heterogeneity of amyotrophic lateral sclerosis: a population based study. J Neurol Neurosurg Psychiatry 2011 Jul;82(7):740-746.

(41) Turner MR, Hardiman O, Benatar M, Brooks BR, Chio A, de Carvalho M, et al. Controversies and priorities in amyotrophic lateral sclerosis. Lancet Neurol 2013 Mar;12(3):310-322.

(42) Murray ME, DeJesus-Hernandez M, Rutherford NJ, Baker M, Duara R, Graff-Radford NR, et al. Clinical and neuropathologic heterogeneity of c9FTD/ALS associated with hexanucleotide repeat expansion in C9ORF72. Acta Neuropathol 2011 Dec;122(6):673-690.

(43) Stewart H, Rutherford NJ, Briemberg H, Krieger C, Cashman N, Fabros M, et al. Clinical and pathological features of amyotrophic lateral sclerosis caused by mutation in the C9ORF72 gene on chromosome 9p. Acta Neuropathol 2012 Jan 7.

(44) Mackenzie IR, Ansorge O, Strong M, Bilbao J, Zinman L, Ang LC, et al. Pathological heterogeneity in amyotrophic lateral sclerosis with FUS mutations: two distinct patterns correlating with disease severity and mutation. Acta Neuropathol 2011 Jul;122(1):87-98.

(45) Cronin S, Hardiman O, Traynor BJ. Ethnic variation in the incidence of ALS: a systematic review. Neurology 2007 Mar 27;68(13):1002-1007.

(46) Alonso A, Logroscino G, Jick SS, Hernan MA. Incidence and lifetime risk of motor neuron disease in the United Kingdom: a population-based study. Eur J Neurol 2009 Jun;16(6):745-751.

(47) Chio A, Logroscino G, Traynor BJ, Collins J, Simeone JC, Goldstein LA, et al. Global epidemiology of amyotrophic lateral sclerosis: a systematic review of the published literature. Neuroepidemiology 2013;41(2):118-130.

(48) Plato CC, Garruto RM, Galasko D, Craig U, Plato M, Gamst A, et al. Amyotrophic Lateral Sclerosis and Parkinsonism-Dementia Complex of Guam: Changing Incidence Rates during the Past 60 Years. Am J Epidemiol 2003;157(2):149-157.

(49) Okamoto K, Kihira T, Kondo T, Kobashi G, Washio M, Sasaki S, et al. Lifestyle Factors and Risk of Amyotrophic Lateral Sclerosis: A Case-Control Study in Japan. Ann Epidemiol 2009;19(6):359-364.

(50) Spencer PS, Palmer VS, Ludolph AC. On the decline and etiology of high-incidence motor system disease in West Papua (southwest New Guinea). Movement disorders : official journal of the Movement Disorder Society, August 01, 2005 2005 20 Suppl 12:S119-S126;20 Suppl 12:S119-S126.

(51) Waring SC, Esteban-Santillan C, Reed DM, Craig U, Labarthe DR, Petersen RC, et al. Incidence of Amyotrophic Lateral Sclerosis and of the Parkinsonism-Dementia Complex of Guam, 1950-1989. Neuroepidemiology 2004 Jul;23(4):192-200.

(52) Steele JC. Parkinsonism-dementia complex of Guam. Movement disorders : official journal of the Movement Disorder Society, August 01, 2005 2005 20 Suppl 12:S99-S107;20 Suppl 12:S99-S107.

(53) Yoshida S, Uebayashi Y, Kihira T, Kohmoto J, Wakayama I, Taguchi S, et al. Epidemiology of motor neuron disease in the Kii Peninsula of Japan, 1989–1993: Active or disappearing focus? J Neurol Sci 1998;155(2):146-155.

(54) Marin B, Couratier P, Preux PM, Logroscino G. Can mortality data be used to estimate amyotrophic lateral sclerosis incidence? Neuroepidemiology 2011;36(1):29-38.

(55) McCombe PA, Henderson RD. Effects of gender in amyotrophic lateral sclerosis. Gender medicine 2010;7(6):557-570.

(56) Gordon PH, Artaud F, Aouba A, Laurent F, Meininger V, Elbaz A. Changing mortality for motor neuron disease in France (1968-2007): an age-period-cohort analysis. Eur J Epidemiol 2011 Sep;26(9):729-737.

(57) Sabatelli M, Madia F, Conte A, Luigetti M, Zollino M, Mancuso I, et al. Natural history of young-adult amyotrophic lateral sclerosis. Neurology 2008 09/16;71(12):876-881.

(58) Brody JA, Grant MD. Age-associated diseases and conditions: implications for decreasing late life morbidity. Aging (Milano) 2001 Apr;13(2):64-67.

(59) Lareau-Trudel E, Fortin E, Gauthier M, Lavoie S, Morissette E, Mathieu J. Epidemiological surveillance of amyotrophic lateral sclerosis in Saguenay region. Can J Neurol Sci 2013 Sep;40(5):705-709.

(60) Armon C. The underestimation of familial ALS and counseling patients with sporadic ALS. Neurology 2014 Jan 7;82(1):13-14.

(61) Byrne S, Elamin M, Bede P, Hardiman O. Absence of consensus in diagnostic criteria for familial neurodegenerative diseases. J Neurol Neurosurg Psychiatry 2012 Apr;83(4):365-367.

(62) Gibson SB, Figueroa KP, Bromberg MB, Pulst SM, Cannon-Albright L. Familial clustering of ALS in a population-based resource. Neurology 2014 Jan 7;82(1):17-22.

(63) Strong MJ, Hudson AJ, Alvord WG. Familial amyotrophic lateral sclerosis, 1850-1989: a statistical analysis of the world literature. Can J Neurol Sci 1991 Feb;18(1):45-58.

(64) Ticozzi N, Tiloca C, Morelli C, Colombrita C, Poletti B, Doretti A, et al. Genetics of familial amyotrophic lateral sclerosis. Arch Ital Biol 2011;149(1):65-82.

(65) Lill CM, Abel O, Bertram L, Al-Chalabi A. Keeping up with genetic discoveries in amyotrophic lateral sclerosis: The ALSoD and ALSGene databases. Amyotroph Lateral Scler 2011 Jul;12(4):238-249.

(66) Corcia P, Camu W, Praline J, Gordon PH, Vourch P, Andres C. The importance of the SMN genes in the genetics of sporadic ALS. Amyotrophic lateral sclerosis : official publication of the World Federation of Neurology Research Group on Motor Neuron Diseases 2009 Oct-Dec;10(5-6):436-440.

(67) Meireles A, Al-Chalabi A. Genetic studies of amyotrophic lateral sclerosis: Controversies and perspectives. Amyotrophic Lat Scler 2009 Feb;10(1):1-14.

(68) Morahan JM, Yu B, Trent RJ, Pamphlett R. Genetic susceptibility to environmental toxicants in ALS. Am J Med Genet B Neuropsychiatr Genet 2007 Oct 5;144B(7):885-890.

(69) J. C. Schymick. The genetics of amyotrophic lateral sclerosis. England: University of Oxford (United Kingdom); 2009.

(70) Gros-Louis F, Gaspar C, Rouleau GA. Genetics of familial and sporadic amyotrophic lateral sclerosis. Biochim Biophys Acta 2006 Nov-Dec;1762(11-12):956-972.

(71) Pasinelli P(, Brown RH(. Molecular biology of amyotrophic lateral sclerosis : insights from genetics. Nature reviews.Neuroscience (Print) 2006;7(9):710-723.

(72) Simpson CL, Al-Chalabi A. Amyotrophic lateral sclerosis as a complex genetic disease. Biochim Biophys Acta 2006 Nov-Dec;1762(11-12):973-985.

(73) Shefner JM, Reaume AG, Flood DG, Scott RW, Kowall NW, Ferrante RJ, et al. Mice lacking cytosolic copper/zinc superoxide dismutase display a distinctive motor axonopathy. Neurology 1999 Oct 12;53(6):1239-1246.

(74) Al-Chalabi A, Jones A, Troakes C, King A, Al-Sarraj S, Van Den Berg LH. The genetics and neuropathology of amyotrophic lateral sclerosis. Acta Neuropathol 2012 September 2012;124(3):339-352.

(75) Gurney ME, Pu H, Chiu AY, Dal Canto MC, Polchow CY, Alexander DD, et al. Motor neuron degeneration in mice that express a human Cu,Zn superoxide dismutase mutation. Science 1994 Jun 17;264(5166):1772-1775.

(76) Mancuso R, Olivan S, Osta R, Navarro X. Evolution of gait abnormalities in SOD1(G93A) transgenic mice. Brain Res 2011 Jul 4.

(77) Peviani M, Caron I, Pizzasegola C, Gensano F, Tortarolo M, Bendotti C. Unraveling the Complexity of Amyotrophic Lateral Sclerosis: Recent Advances from the Transgenic Mutant SOD1 Mice. CNS & NEUROLOGICAL DISORDERS-DRUG TARGETS 2010;9(4):491-503.

(78) Turner B, Talbot K. Transgenics, toxicity and therapeutics in rodent models of mutant SOD1-mediated familial ALS. Prog Neurobiol 2008;85(1):94-134.

(79) Rosen DR, Bowling AC, Patterson D, Usdin TB, Sapp P, Mezey E, et al. A frequent ala 4 to val superoxide dismutase-1 mutation is associated with a rapidly progressive familial amyotrophic lateral sclerosis. Hum Mol Genet 1994 Jun;3(6):981-987.

(80) Rosen DR. Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. Nature 1993 Jul 22;364(6435):362.

(81) Arisato T, Okubo R, Arata H, Abe K, Fukada K, Sakoda S, et al. Clinical and pathological studies of familial amyotrophic lateral sclerosis (FALS) with SOD1 H46R mutation in large Japanese families. Acta Neuropathol 2003 Dec;106(6):561-568.

(82) Ferrucci M, Fulceri F, Toti L, Soldani P, Siciliano G, Paparelli A, et al. Protein clearing pathways in ALS. Arch Ital Biol 2011;149(1):121-149.

(83) Shi P, Gal J, Kwinter DM, Liu X, Zhu H. Mitochondrial dysfunction in amyotrophic lateral sclerosis. Biochimica et biophysica acta, January 01 2010;NIHMS141753; PMC2790551(1):45-51.

(84) Dormann D, Haass C. TDP-43 and FUS: a nuclear affair. Trends Neurosci 2011 Jun 21.

(85) Lagier-Tourenne C, Cleveland DW. Rethinking ALS: the FUS about TDP-43. Cell 2009 Mar 20;136(6):1001-1004.

(86) Lai SL, Abramzon Y, Schymick JC, Stephan DA, Dunckley T, Dillman A, et al. FUS mutations in sporadic amyotrophic lateral sclerosis. Neurobiol Aging 2011 Mar;32(3):550.e1-550.e4.

(87) Kanekura K, Suzuki H, Aiso S, Matsuoka M. ER stress and unfolded protein response in amyotrophic lateral sclerosis. Molecular neurobiology, April 01 2009;39(2):81-89.

(88) Soo KY, Atkin JD, Farg M, Walker AK, Horne MK, Nagley P. Bim links ER stress and apoptosis in cells expressing mutant SOD1 associated with amyotrophic lateral sclerosis. PLoS One 2012;7(4):e35413.

(89) Hetz C, Thielen P, Matus S, Nassif M, Court F, Kiffin R, et al. XBP-1 deficiency in the nervous system protects against amyotrophic lateral sclerosis by increasing autophagy. Genes Dev 2009 Oct 1;23(19):2294-2306.

(90) Uccelli R, Binazzi A, Altavista P, Belli S, Comba P, Mastrantonio M, et al. Geographic distribution of amyotrophic lateral sclerosis through motor neuron disease mortality data. Eur J Epidemiol 2007;22(11):781-790.

(91) Noonan CW, White MC, Thurman D, Wong LY. Temporal and geographic variation in United States motor neuron disease mortality, 1969-1998. Neurology 2005 Apr 12;64(7):1215-1221.

(92) Doi Y, Yokoyama T, Tango T, Takahashi K, Fujimoto K, Nakano I. Temporal trends and geographic clusters of mortality from amyotrophic lateral sclerosis in Japan, 1995-2004. J Neurol Sci 2010 Nov 15;298(1-2):78-84.

(93) Chio A, Meineri P, Tribolo A, Schiffer D. Risk factors in motor neuron disease: a case-control study. Neuroepidemiology 1991;10(4):174-184.

(94) Gundogdu B, Al-Lahham T, Kadlubar F, Spencer H, Rudnicki SA. Racial differences in motor neuron disease. Amyotroph Lateral Scler Frontotemporal Degener 2013 Sep 25.

(95) Ogaki K, Li Y, Atsuta N, Tomiyama H, Funayama M, Watanabe H, et al. Analysis of C9orf72 repeat expansion in 563 Japanese patients with amyotrophic lateral sclerosis. Neurobiol Aging 2012 Jun 21.

(96) Ishiura H, Takahashi Y, Mitsui J, Yoshida S, Kihira T, Kokubo Y, et al. C9ORF72 Repeat Expansion in Amyotrophic Lateral Sclerosis in the Kii Peninsula of JapanC9ORF72 Repeat Expansion in ALS. Arch Neurol 2012 Jun 4:1-5.

(97) Konno T, Shiga A, Tsujino A, Sugai A, Kato T, Kanai K, et al. Japanese amyotrophic lateral sclerosis patients with GGGGCC hexanucleotide repeat expansion in C9ORF72. J Neurol Neurosurg Psychiatry 2013 Apr;84(4):398-401.

(98) Zou ZY, Wang XN, Liu MS, Sun Q, Li XG, Cui LY, et al. Identification of a novel missense mutation in angiogenin in a Chinese amyotrophic lateral sclerosis cohort. Amyotroph Lateral Scler 2012 Jan 31.

(99) Tsai CP, Soong BW, Tu PH, Lin KP, Fuh JL, Tsai PC, et al. A hexanucleotide repeat expansion in C9ORF72 causes familial and sporadic ALS in Taiwan. Neurobiol Aging 2012 Sep;33(9):2232.e11-2232.e18.

(100) Migliore L, Coppede F. Genetics, environmental factors and the emerging role of epigenetics in neurodegenerative diseases. Mutat Res 2009 Jul 10;667(1-2):82-97.

(101) Callaghan B, Feldman D, Gruis K, Feldman E. The Association of Exposure to Lead, Mercury, and Selenium and the Development of Amyotrophic Lateral Sclerosis and the Epigenetic Implications. NEURODEGENERATIVE DISEASES 2011;8(1-2):1-8.

(102) Figueroa-Romero C, Hur J, Bender DE, Delaney CE, Cataldo MD, Smith AL, et al. Identification of epigenetically altered genes in sporadic amyotrophic lateral sclerosis. PLoS One 2012;7(12):e52672.

(103) Aggarwal A, Nicholson G. Age dependent penetrance of three different superoxide dismutase 1 (sod 1) mutations. Int J Neurosci 2005 Aug;115(8):1119-1130.

(104) Paolino E, Granieri E, Tola MR, Rosati G. Conjugal amyotrophic lateral sclerosis. Ann Neurol 1983 Dec;14(6):699.

(105) Cornblath DR, Kurland LT, Boylan KB, Morrison L, Radhakrishnan K, Montgomery M. Conjugal amyotrophic lateral sclerosis: report of a young married couple. Neurology 1993 Nov;43(11):2378-2380.

(106) Poloni M, Micheli A, Facchetti D, Mai R, Ceriani F, Cattalini C. Conjugal amyotrophic lateral sclerosis: toxic clustering or change? Ital J Neurol Sci 1997 Apr;18(2):109-112.

(107) Rachele MG, Mascia V, Tacconi P, Dessi N, Marrosu F, Giagheddu M. Conjugal amyotrophic lateral sclerosis: a report on a couple from Sardinia, Italy. Ital J Neurol Sci 1998 Apr;19(2):97-100.

(108) Corcia P, Jafari-Schluep HF, Lardillier D, Mazyad H, Giraud P, Clavelou P, et al. A clustering of conjugal amyotrophic lateral sclerosis in southeastern France. Arch Neurol 2003 Apr;60(4):553-557.

(109) Stipa G, Taiuti R, de Scisciolo G, Arnetoli G, Tredici MR, Biondi N, et al. Sporadic amyotrophic lateral sclerosis as an infectious disease: a possible role of cyanobacteria? Med Hypotheses 2006;67(6):1363-1371.

(110) Godeiro Jr C, Oliveira AS, Felicio AC, Chieia MA, Gabbai AA. Conjugal amyotrophic lateral sclerosis in Brazil. Arq Neuropsiquiatr 2009 Dec;67(4):1045-1048.

(111) Wills AM, Cronin S, Slowik A, Kasperaviciute D, Van Es MA, Morahan JM, et al. A large-scale international meta-analysis of paraoxonase gene polymorphisms in sporadic ALS. Neurology 2009 Jul 7;73(1):16-24.

(112) Lambrechts D, Poesen K, Fernandez-Santiago R, Al-Chalabi A, Del Bo R, Van Vught P, et al. Meta-analysis of vascular endothelial growth factor variations in amyotrophic lateral sclerosis: increased susceptibility in male carriers of the-2578AA genotype. J Med Genet 2009;46(12):840-846.

(113) Praline J, Blasco H, Vourc'h P, Rat V, Gendrot C, Camu W, et al. Study of the HFE gene common polymorphisms in French patients with sporadic amyotrophic lateral sclerosis. J Neurol Sci 2012 Jun 15;317(1-2):58-61.

(114) van Rheenen W, Diekstra FP, van Doormaal PT, Seelen M, Kenna K, McLaughlin R, et al. H63D polymorphism in HFE is not associated with amyotrophic lateral sclerosis. Neurobiol Aging 2013 May;34(5):1517.e5-1517.e7.

(115) Sutedja NA, Sinke RJ, Van Vught PW, Van der Linden MW, Wokke JH, Van Duijn CM, et al. The association between H63D mutations in HFE and amyotrophic lateral sclerosis in a Dutch population. Arch Neurol 2007 Jan;64(1):63-67.

(116) Restagno G, Lombardo F, Ghiglione P, Calvo A, Cocco E, Sbaiz L, et al. HFE H63D polymorphism is increased in patients with amyotrophic lateral sclerosis of Italian origin. J NEUROL NEUROSURG PSYCHIATRY 2007 03;78(3):327-327.

(117) Wang XS, Lee S, Simmons Z, Boyer P, Scott K, Liu W, et al. Increased incidence of the Hfe mutation in amyotrophic lateral sclerosis and related cellular consequences. J Neurol Sci 2004 Dec 15;227(1):27-33.

(118) Yen AA, Simpson EP, Henkel JS, Beers DR, Appel SH. HFE mutations are not strongly associated with sporadic ALS. Neurology 2004 May 11;62(9):1611-1612.

(119) Belzil VV, Bauer PO, Prudencio M, Gendron TF, Stetler CT, Yan IK, et al. Reduced C9orf72 gene expression in c9FTD/ALS is caused by histone trimethylation, an epigenetic event detectable in blood. Acta Neuropathol 2013 Dec;126(6):895-905.

(120) Johnson F, Atchison W. The role of environmental mercury, lead and pesticide exposure in development of amyotrophic lateral sclerosis. Neurotoxicology 2009 Sep;30(5):761-765.

(121) Caban-Holt A, Mattingly M, Cooper G, Schmitt FA. Neurodegenerative memory disorders: a potential role of environmental toxins. Neurol Clin 2005 May;23(2):485-521.

(122) Wicklund M. Amyotrophic lateral sclerosis: Possible role of environmental influences. Neurol Clin 2005;23(2):461-+.

(123) Bonvicini F, Marcello N, Mandrioli J, Pietrini V, Vinceti M. Exposure to pesticides and risk of amyotrophic lateral sclerosis: a population-based case-control study. Ann Ist Super Sanita 2010;46(3):284-287.

(124) Govoni V, Granieri E, Fallica E, Casetta I. Amyotrophic lateral sclerosis, rural environment and agricultural work in the Local Health District of Ferrara, Italy, in the years 1964-1998. J Neurol 2005 Nov;252(11):1322-1327.

(125) Gunnarsson LG, Lindberg G, Soderfeldt B, Axelson O. Amyotrophic lateral sclerosis in Sweden in relation to occupation. Acta Neurol Scand 1991 Jun;83(6):394-398.

(126) Chen H, Richard M, Sandler DP, Umbach DM, Kamel F. Head injury and amyotrophic lateral sclerosis. Am J Epidemiol 2007 Oct 1;166(7):810-816.

(127) Armon C. Sports and trauma in amyotrophic lateral sclerosis revisited. J Neurol Sci 2007 Nov 15;262(1-2):45-53.

(128) Cruz DC, Nelson LM, McGuire V, Longstreth WT,Jr. Physical trauma and family history of neurodegenerative diseases in amyotrophic lateral sclerosis: a population-based case-control study. Neuroepidemiology 1999;18(2):101-110.

(129) Kurland LT, Radhakrishnan K, Smith GE, Armon C, Nemetz PN. Mechanical trauma as a risk factor in classic amyotrophic lateral sclerosis: Lack of epidemiologic evidence. J Neurol Sci 1992;113(2):133-143.

(130) Fang F, Kwee LC, Allen KD, Umbach DM, Ye W, Watson M, et al. Association between blood lead and the risk of amyotrophic lateral sclerosis. Am J Epidemiol 2010 May 15;171(10):1126-1133.

(131) Wang H, O'Reilly ÉJ, Weisskopf MG, Logroscino G, McCullough ML, Thun MJ, et al. Smoking and risk of amyotrophic lateral sclerosis: a pooled analysis of 5 prospective cohorts. Arch Neurol 2011;68(2):207-213.

(132) Fang F, Ye W. Smoking may be Considered an Established Risk Factor for Sporadic Als. Neurology 2010;74(23):1927-1927.

(133) Li CY, Sung FC. Association between occupational exposure to power frequency electromagnetic fields and amyotrophic lateral sclerosis: a review. Am J Ind Med 2003 Feb;43(2):212-220.

(134) Zhou H, Chen G, Chen C, Yu Y, Xu Z. Association between extremely low-frequency electromagnetic fields occupations and amyotrophic lateral sclerosis: a meta-analysis. PLoS One 2012;7(11):e48354.

(135) Harwood CA, McDermott CJ, Shaw PJ. Physical activity as an exogenous risk factor in motor neuron disease (MND): a review of the evidence. Amyotroph Lateral Scler 2009 Aug;10(4):191-204.

(136) Veldink J, Kalmijn S, Groeneveld G, Titulaer M, Wokke J, van den Berg L. Physical activity and the association with sporadic ALS. Neurology 2005;64(2):241-245.

(137) Esik O, Vönöczky K, Lengyel Z, Sáfrány G, Trón L. Characteristics of radiogenic lower motor neurone disease, a possible link with a preceding viral infection. Spinal cord : the official journal of the International Medical Society of Paraplegia, February 01 2004;42(2):99-105.

(138) Belleroche J, Orrell R, Virgo L. Amyotrophic lateral sclerosis: Recent advances in understanding disease mechanisms. J Neuropathol Exp Neurol 1996;55(7):747-757.

(139) Malek AM, Barchowsky A, Bowser R, Youk A, Talbott EO. Pesticide exposure as a risk factor for amyotrophic lateral sclerosis: A meta-analysis of epidemiological studies: Pesticide exposure as a risk factor for ALS. Environ Res 2012 Jul 19.

(140) Kamel F, Umbach DM, Bedlack RS, Richards M, Watson M, Alavanja MC, et al. Pesticide exposure and amyotrophic lateral sclerosis. Neurotoxicology 2012 Apr 12.

(141) McGuire V, Longstreth WT,Jr, Nelson LM, Koepsell TD, Checkoway H, Morgan MS, et al. Occupational exposures and amyotrophic lateral sclerosis. A population-based case-control study. Am J Epidemiol 1997 Jun 15;145(12):1076-1088.

(142) Chancellor AM, Slattery JM, Fraser H, Warlow CP. Risk factors for motor neuron disease: a case-control study based on patients from the Scottish Motor Neuron Disease Register. J Neurol Neurosurg Psychiatry 1993 Nov;56(11):1200-1206.

(143) Fang F, Quinlan P, Ye W, Barber MK, Umbach DM, Sandler DP, et al. Workplace exposures and the risk of amyotrophic lateral sclerosis. Environ Health Perspect 2009 Sep;117(9):1387-1392.

(144) Qureshi MM, Hayden D, Urbinelli L, Ferrante K, Newhall K, Myers D, et al. Analysis of factors that modify susceptibility and rate of progression in amyotrophic lateral sclerosis (ALS). Amyotroph Lateral Scler 2006 Sep;7(3):173-182.

(145) Savettieri G, Salemi G, Arcara A, Cassata M, Castiglione MG, Fierro B. A case-control study of amyotrophic lateral sclerosis. Neuroepidemiology 1991;10(5-6):242-245.

(146) Morahan JM, Pamphlett R. Amyotrophic lateral sclerosis and exposure to environmental toxins: an Australian case-control study. Neuroepidemiology 2006;27(3):130-135.

(147) Gunnarsson LG, Bodin L, Soderfeldt B, Axelson O. A case-control study of motor neurone disease: its relation to heritability, and occupational exposures, particularly to solvents. Br J Ind Med 1992 Nov;49(11):791-798.

(148) Furby A, Beauvais K, Kolev I, Rivain JG, Sebille V. Rural environment and risk factors of amyotrophic lateral sclerosis: a case-control study. J Neurol 2010 May;257(5):792-798.

(149) Weisskopf MG, Morozova N, O'Reilly E, McCullough ML, Calle EE, Thun MJ, et al. Prospective study of chemical exposures and amyotrophic lateral sclerosis. J NEUROL NEUROSURG PSYCHIATRY 2009 05;80(5):558-561.

(150) Park R, Schulte P, Bowman J, Walker J, Bondy S, Yost M, et al. Potential occupational risks for neurodegenerative diseases. Am J Ind Med 2005;48(1):63-77.

(151) Buckley J, Warlow C, Smith P, Hilton-Jones D, Irvine S, Tew JR. Motor neuron disease in England and Wales, 1959-1979. J Neurol Neurosurg Psychiatry 1983 Mar;46(3):197-205.

(152) Malek AM, Barchowsky A, Bowser R, Heiman-Patterson T, Lacomis D, Rana S, et al. Environmental and Occupational Risk Factors for Amyotrophic Lateral Sclerosis: A Case-Control Study. Neurodegener Dis 2013 Nov 12.

(153) Iwami O, Watanabe T, Moon C, Nakatsuka H, Ikeda M. Motor Neuron Disease on the Kii Peninsula of Japan: Excess Manganese Intake from Food Coupled with Low Magnesium in Drinking Water as a Risk Factor. Sci Total Environ 1994 13 Jun;149(1):121 p.

(154) Vinceti M, Bottecchi I, Fan A, Finkelstein Y, Mandrioli J. Are environmental exposures to selenium, heavy metals, and pesticides risk factors for amyotrophic lateral sclerosis? Rev Environ Health 2012;27(1):19-41.

(155) Ahmed A, Wicklund MP. Amyotrophic lateral sclerosis: what role does environment play? Neurol Clin 2011 Aug;29(3):689-711.

(156) Mitchell JD. Heavy metals and trace elements in amyotrophic lateral sclerosis. Neurol Clin 1987 Feb;5(1):43-60.

(157) Mitchell JD, East BW, Harris IA, Pentland B. Manganese, selenium and other trace elements in spinal cord, liver and bone in motor neurone disease. Eur Neurol 1991;31(1):7-11.

(158) Valentine JS, Doucette PA, Zittin Potter S. Copper-zinc superoxide dismutase and amyotrophic lateral sclerosis. Annual review of biochemistry, Jan 01 2005;74:563-593.

(159) Trumbull KA, Beckman JS. A role for copper in the toxicity of zinc-deficient superoxide dismutase to motor neurons in amyotrophic lateral sclerosis. Antioxid Redox Signal 2009 Jul;11(7):1627-1639.

(160) Bowman AB, Kwakye GF, Hernandez EH, Aschner M. Role of manganese in neurodegenerative diseases. J Trace Elem Med Biol 2011 Dec;25(4):191-203.

(161) Kurlander HM(, Patten BM(. Metals in spinal cord tissue of patients dying of motor neuron disease. Ann Neurol 1979;6(1):21-24.

(162) Groeneveld GJ, de Leeuw van Weenen J, van Muiswinkel FL, Veldman H, Veldink JH, Wokke JH, et al. Zinc amplifies mSOD1-mediated toxicity in a transgenic mouse model of amyotrophic lateral sclerosis. Neurosci Lett 2003 Dec 11;352(3):175-178.

(163) Ermilova IP, Ermilov VB, Levy M, Ho E, Pereira C, Beckman JS. Protection by dietary zinc in ALS mutant G93A SOD transgenic mice. Neurosci Lett 2005 Apr 29;379(1):42-46.

(164) Vinceti M, Bonvicini F, Bergomi M, Malagoli C. Possible involvement of overexposure to environmental selenium in the etiology of amyotrophic lateral sclerosis: a short review. Ann Ist Super Sanita 2010;46(3):279-283.

(165) Fondell E, O'Reilly EJ, Fitzgerald KC, Falcone GJ, McCullough ML, Park Y, et al. Magnesium intake and risk of amyotrophic lateral sclerosis: Results from five large cohort studies. Amyotroph Lateral Scler Frontotemporal Degener 2013 Jun 18.

(166) Praline J, Guennoc AM, Limousin N, Hallak H, de Toffol B, Corcia P. ALS and mercury intoxication: a relationship? Clin Neurol Neurosurg 2007 Dec;109(10):880-883.

(167) Pierce-Ruhland R, Patten BM. Repeat study of antecedent events in motor neuron disease. Ann Clin Res 1981 Apr;13(2):102-107.

(168) Mano Y, Takayanagi T, Abe T, Takizawa Y. Amyotrophic lateral sclerosis and mercury--preliminary report. Rinsho Shinkeigaku 1990 Nov;30(11):1275-1277.

(169) Moriwaka F, Satoh H, Ejima A, Watanabe C, Tashiro K, Hamada T, et al. Mercury and selenium contents in amyotrophic lateral sclerosis in Hokkaido, the northernmost island of Japan. J Neurol Sci 1993 Aug;118(1):38-42.

(170) Huss A, Spoerri A, Egger M, Roosli M, Swiss National Cohort Study. Residence near power lines and mortality from neurodegenerative diseases: longitudinal study of the Swiss population. Am J Epidemiol 2009 Jan 15;169(2):167-175.

(171) Consales C, Merla C, Marino C, Benassi B. Electromagnetic fields, oxidative stress, and neurodegeneration. Int J Cell Biol 2012;2012:683897.

(172) ALPERS BJ, FARMER RA. Role of repeated trauma by pneumatic drill in production of amyotrophic lateral sclerosis. Arch Neurol Psychiatry 1949 Aug;62(2):178-182.

(173) McKee AC, Cantu RC, Nowinski CJ, Hedley-Whyte ET, Gavett BE, Budson AE, et al. Chronic traumatic encephalopathy in athletes: progressive tauopathy after repetitive head injury. J Neuropathol Exp Neurol 2009 Jul;68(7):709-735.

(174) McKee AC, Stein TD, Nowinski CJ, Stern RA, Daneshvar DH, Alvarez VE, et al. The spectrum of disease in chronic traumatic encephalopathy. Brain 2013 Jan;136(Pt 1):43-64.

(175) Turner MR, Abisgold J, Yeates DG, Talbot K, Goldacre MJ. Head and other physical trauma requiring hospitalisation is not a significant risk factor in the development of ALS. J Neurol Sci 2010 Jan 15;288(1-2):45-48.

(176) Beghi E, Logroscino G, Chio A, Hardiman O, Millul A, Mitchell D, et al. Amyotrophic lateral sclerosis, physical exercise, trauma and sports: results of a population-based pilot case-control study. Amyotroph Lateral Scler 2010 May 3;11(3):289-292.

(177) Rosenbohm A, Kassubek J, Weydt P, Marroquin N, Volk AE, Kubisch C, et al. Can lesions to the motor cortex induce amyotrophic lateral sclerosis? J Neurol 2013 Nov 20.

(178) Valenti M, Pontieri FE, Conti F, Altobelli E, Manzoni T, Frati L. Amyotrophic lateral sclerosis and sports: a case-control study. Eur J Neurol 2005 Mar;12(3):223-225.

(179) Longstreth WT, McGuire V, Koepsell TD, Wang Y, van Belle G. Risk of amyotrophic lateral sclerosis and history of physical activity: a population-based case-control study. Arch Neurol 1998 Feb;55(2):201-206.

(180) Chio A, Calvo A, Dossena M, Ghiglione P, Mutani R, Mora G. ALS in Italian professional soccer players: the risk is still present and could be soccer-specific. Amyotroph Lateral Scler 2009 Aug;10(4):205-209.

(181) Vanacore N, Binazzi A, Bottazzi M, Belli S. Amyotrophic lateral sclerosis in an Italian professional soccer player. Parkinsonism Relat Disord 2006 Jun;12(5):327-329.

(182) Belli S, Vanacore N. Proportionate mortality of Italian soccer players: is amyotrophic lateral sclerosis an occupational disease? Eur J Epidemiol 2005;20(3):237-242.

(183) Wicks P, Ganesalingham J, Collin C, Prevett M, Leigh NP, Al-Chalabi A. Three soccer playing friends with simultaneous amyotrophic lateral sclerosis. Amyotrophic Lat Scler 2007;8(3):177-179.

(184) Abel EL. Football increases the risk for Lou Gehrig's disease, amyotrophic lateral sclerosis. Percept Mot Skills 2007 Jun;104(3 Pt 2):1251-1254.

(185) Gallo V, Bueno-De-Mesquita H, Vermeulen R, Andersen PM, Kyrozis A, Linseisen J, et al. Smoking and risk for amyotrophic lateral sclerosis: analysis of the EPIC cohort. Ann Neurol 2009 04;65(4):378-385.

(186) Kamel F, Umbach D, Munsat T, Shefner J, Hu H, Sandler D. Lead exposure and amyotrophic lateral sclerosis. Epidemiology 2002;13(3):311-319.

(187) Sutedja NA, Veldink JH, Fischer K, Kromhout H, Wokke JH, Huisman MH, et al. Lifetime occupation, education, smoking, and risk of ALS. Neurology 2007 10/09;69(15):1508-1514.

(188) Werneck LC, Bezerra R, Silveira Neto O, Scola RH. A clinical epidemiological study of 251 cases of amyotrophic lateral sclerosis in the south of Brazil. Arq Neuropsiquiatr 2007 Jun;65(2A):189-195.

(189) Gunnarsson LG, Dahlbom K, Strandman E. Motor neuron disease and dementia reported among 13 members of a single family. Acta Neurol Scand 1991 Nov;84(5):429-433.

(190) Vanacore N, Cocco P, Fadda D, Dosemeci M. Job strain, hypoxia and risk of amyotrophic lateral sclerosis: Results from a death certificate study. Amyotroph Lateral Scler 2010 Oct;11(5):430-434.

(191) LEEDER J. Smoke, fire and Lou Gehrig's disease. Jul. 21 2007.

(192) Brockington A, Wharton SB, Fernando M, Gelsthorpe CH, Baxter L, Ince PG, et al. Expression of vascular endothelial growth factor and its receptors in the central nervous system in amyotrophic lateral sclerosis. J Neuropathol Exp Neurol 2006 Jan;65(1):26-36.

(193) Moreau C, Devos D, Brunaud-Danel V, Defebvre L, Perez T, Destee A, et al. Paradoxical response of VEGF expression to hypoxia in CSF of patients with ALS. J Neurol Neurosurg Psychiatry 2006 Feb;77(2):255-257.

(194) Moreau C, Gosset P, Kluza J, Brunaud-Danel V, Lassalle P, Marchetti P, et al. Deregulation of the hypoxia inducible factor-1alpha pathway in monocytes from sporadic amyotrophic lateral sclerosis patients. Neuroscience 2011 Jan 13;172:110-117.

(195) Weisskopf MG, McCullough ML, Morozova N, Calle EE, Thun MJ, Ascherio A. Prospective study of occupation and amyotrophic lateral sclerosis mortality. Am J Epidemiol 2005 Dec 15;162(12):1146-1152.

(196) Scarmeas N, Shih T, Stern Y, Ottman R, Rowland LP. Premorbid weight, body mass, and varsity athletics in ALS. Neurology 2002 Sep 10;59(5):773-775.

(197) O'Reilly EJ, Wang H, Weisskopf MG, Fitzgerald KC, Falcone G, McCullough ML, et al. Premorbid body mass index and risk of amyotrophic lateral sclerosis. Amyotroph Lateral Scler Frontotemporal Degener 2013 Apr;14(3):205-211.

(198) Sutedja NA, van der Schouw YT, Fischer K, Sizoo EM, Huisman MH, Veldink JH, et al. Beneficial vascular risk profile is associated with amyotrophic lateral sclerosis. J Neurol Neurosurg Psychiatry 2011 Jun;82(6):638-642.

(199) Gallo V, Wark PA, Jenab M, Pearce N, Brayne C, Vermeulen R, et al. Prediagnostic body fat and risk of death from amyotrophic lateral sclerosis: the EPIC cohort. Neurology 2013 Feb 26;80(9):829-838.

(200) Paganoni S, Deng J, Jaffa M, Cudkowicz ME, Wills AM. Body mass index, not dyslipidemia, is an independent predictor of survival in amyotrophic lateral sclerosis. Muscle Nerve 2011 Jul;44(1):20-24.

(201) Dupuis L, Corcia P, Fergani A, Gonzalez De Aguilar JL, Bonnefont-Rousselot D, Bittar R, et al. Dyslipidemia is a protective factor in amyotrophic lateral sclerosis. Neurology 2008 Mar 25;70(13):1004-1009.

(202) Nelson L, Matkin C, Longstreth W, McGuire V. Population-based case-control study of amyotrophic lateral sclerosis in western Washington Sate. II. Diet. Am J Epidemiol 2000;151(2):164-173.

(203) Kamel F, Umbach DM, Munsat TL, Shefner JM, Sandler DP. Association of Cigarette Smoking with Amyotrophic Lateral Sclerosis. Neuroepidemiology 1999 Jul;18(4):194-202.

(204) Fang F, Bellocco R, Heman M, Ye W. Smoking, Snuff Dipping and the Risk of Amyotrophic Lateral Sclerosis - A Prospective Cohort Study. Neuroepidemiology 2006 Dec;27(4):217-221.

(205) Berg L, de Jong S, Huisman M, Van der Kooi A, De Visser M, Schelhaas H, et al. Smoking, Alcohol Consumption and the Risk of Amyotrophic Lateral Sclerosis: A Population-Based Study. Neurology 2011;76(9):A115-A115.

(206) Alonso A, Logroscino G, Jick S, Hernan M. Association of smoking with amyotrophic lateral sclerosis risk and survival in men and women: a prospective study. BMC NEUROLOGY 2010;10.

(207) Schmidt S, Kwee LC, Allen KD, Oddone EZ. Association of ALS with head injury, cigarette smoking and APOE genotypes. J Neurol Sci 2010 Apr 15;291(1-2):22-29.

(208) Wang H, Weisskopf M, O'Reilly E, Logroscino G, McCullough M, Schatzkin A, et al. Prospective studies on smoking and risk off amyotrophic lateral sclerosis. Neurology 2008;70(11):A190-A190.

(209) Weisskopf MG, McCullough ML, Calle EE, Thun MJ, Cudkowicz M, Ascherio A. Prospective study of cigarette smoking and amyotrophic lateral sclerosis. Am J Epidemiol 2004 Jul 1;160(1):26-33.

(210) Armon C. Smoking may be considered an established risk factor for sporadic ALS. Neurology 2009;73(20):1693-1698.

(211) Daviglus ML, Plassman BL, Pirzada A, Bell CC, Bowen PE, Burke JR, et al. Risk factors and preventive interventions for Alzheimer disease: state of the science. Arch Neurol 2011 Sep;68(9):1185-1190.

(212) de Jong SW, Huisman MH, Sutedja NA, van der Kooi AJ, de Visser M, Schelhaas HJ, et al. Smoking, Alcohol Consumption, and the Risk of Amyotrophic Lateral Sclerosis: A Population-based Study. Am J Epidemiol 2012 Jul 11.

(213) Beghi E, Pupillo E, Messina P, Giussani G, Chio A, Zoccolella S, et al. Coffee and amyotrophic lateral sclerosis: a possible preventive role. Am J Epidemiol 2011 Nov 1;174(9):1002-1008.

(214) Morozova N, Weisskopf M, McCullough M, Munger K, Calle E, Thun M, et al. Diet and amyotrophic lateral sclerosis. Epidemiology 2008;19(2):324-337.

(215) Bonnefont-Rousselot D, Lacomblez L, Jaudon M, Lepage S, Salachas F, Bensimon G, et al. Blood oxidative stress in amyotrophic lateral sclerosis. J Neurol Sci 2000 Sep 1;178(1):57-62.

(216) Oteiza PI, Uchitel OD, Carrasquedo F, Dubrovski AL, Roma JC, Fraga CG. Evaluation of antioxidants, protein, and lipid oxidation products in blood from sporadic amyotrophic lateral sclerosis patients. Neurochem Res 1997 Apr;22(4):535-539.

(217) Hall ED, Andrus PK, Oostveen JA, Fleck TJ, Gurney ME. Relationship of oxygen radical-induced lipid peroxidative damage to disease onset and progression in a transgenic model of familial ALS. J Neurosci Res 1998 Jul 1;53(1):66-77.

(218) Kaal EC, Veldman H, Sodaar P, Joosten EA, Dop Bar PR. Oxidant treatment causes a dose-dependent phenotype of apoptosis in cultured motoneurons. J Neurosci Res 1998 Dec 15;54(6):778-786.

(219) Okamoto K, Kihira T, Kobashi G, Washio M, Sasaki S, Yokoyama T, et al. Fruit and vegetable intake and risk of amyotrophic lateral sclerosis in Japan. Neuroepidemiology 2009;32(4):251-256.

(220) Veldink JH, Kalmijn S, Groeneveld G, Wunderink W, Koster A, de Vries J, et al. Intake of polyunsaturated fatty acids and vitamin E reduces the risk of developing amyotrophic lateral sclerosis. J NEUROL NEUROSURG PSYCHIATRY 2007 04;78(4):367-371.

(221) Wang H, O'Reilly EJ, Weisskopf MG, Logroscino G, McCullough ML, Schatzkin A, et al. Vitamin E intake and risk of amyotrophic lateral sclerosis: a pooled analysis of data from 5 prospective cohort studies. Am J Epidemiol 2011 Mar 15;173(6):595-602.

(222) Michal Freedman D, Kuncl RW, Weinstein SJ, Malila N, Virtamo J, Albanes D. Vitamin E serum levels and controlled supplementation and risk of amyotrophic lateral sclerosis. Amyotroph Lateral Scler Frontotemporal Degener 2013 Jan 4.

(223) Desnuelle C, Dib M, Garrel C, Favier A. A double-blind, placebo-controlled randomized clinical trial of alpha-tocopherol (vitamin E) in the treatment of amyotrophic lateral sclerosis. ALS riluzole-tocopherol Study Group. Amyotroph Lateral Scler Other Motor Neuron Disord 2001 Mar;2(1):9-18.

(224) Graf M, Ecker D, Horowski R, Kramer B, Riederer P, Gerlach M, et al. High dose vitamin E therapy in amyotrophic lateral sclerosis as add-on therapy to riluzole: results of a placebo-controlled double-blind study. J Neural Transm 2005 May;112(5):649-660.

(225) Galbussera A, Tremolizzo L, Brighina L, Testa D, Lovati R, Ferrarese C, et al. Vitamin E intake and quality of life in amyotrophic lateral sclerosis patients: a follow-up case series study. Neurol Sci 2006 Jul;27(3):190-193.

(226) Vandenberghe N, Leveque N, Corcia P, Brunaud-Danel V, Salort-Campana E, Besson G, et al. Cerebrospinal fluid detection of enterovirus genome in ALS: a study of 242 patients and 354 controls. Amyotroph Lateral Scler 2010 May 3;11(3):277-282.

(227) Cermelli C, Vinceti M, Beretti F, Pietrini V, Nacci G, Pietrosemoli P, et al. Risk of sporadic amyotrophic lateral sclerosis associated with seropositivity for herpesviruses and echovirus-7. Eur J Epidemiol 2003;18(2):123-127.

(228) Berger MM, Kopp N, Vital C, Redl B, Aymard M, Lina B. Detection and cellular localization of enterovirus RNA sequences in spinal cord of patients with ALS. Neurology 2000 Jan 11;54(1):20-25.

(229) Jubelt B. Motor neuron diseases and viruses: poliovirus, retroviruses, and lymphomas. Curr Opin Neurol Neurosurg 1992 Oct;5(5):655-658.

(230) Jubelt B, Lipton HL. ALS: persistent scientists do not find persisting enteroviruses. Neurology 2004 Apr 27;62(8):1250-1251.

(231) Nix WA, Berger MM, Oberste MS, Brooks BR, McKenna-Yasek DM, Brown RH,Jr, et al. Failure to detect enterovirus in the spinal cord of ALS patients using a sensitive RT-PCR method. Neurology 2004 Apr 27;62(8):1372-1377.

(232) Rentzos M, Evangelopoulos E, Sereti E, Zouvelou V, Marmara S, Alexakis T, et al. Alterations of T cell subsets in ALS: a systemic immune activation? Acta Neurol Scand 2011 Jun 9.

(233) Alfahad T, Nath A. Retroviruses and amyotrophic lateral sclerosis. Antiviral Res 2013 May 23.

(234) Garbuzova-Davis S, Rodrigues MC, Hernandez-Ontiveros DG, Louis MK, Willing AE, Borlongan CV, et al. Amyotrophic lateral sclerosis: a neurovascular disease. Brain Res 2011 Jun 29;1398:113-125.

(235) Rodrigues MC, Hernandez-Ontiveros DG, Louis MK, Willing AE, Borlongan CV, Sanberg PR, et al. Neurovascular aspects of amyotrophic lateral sclerosis. Int Rev Neurobiol 2012;102:91-106.

(236) Terry PD, Kamel F, Umbach DM, Lehman TA, Hu H, Sandler DP, et al. VEGF promoter haplotype and amyotrophic lateral sclerosis (ALS). Journal of neurogenetics 2004 Apr-Jun;18(2):429-434.

(237) Barbeito AG, Martinez-Palma L, Vargas MR, Pehar M, Manay N, Beckman JS, et al. Lead exposure stimulates VEGF expression in the spinal cord and extends survival in a mouse model of ALS. Neurobiol Dis 2010 Mar;37(3):574-580.

(238) Wang Y, Mao XO, Xie L, Banwait S, Marti HH, Greenberg DA, et al. Vascular endothelial growth factor overexpression delays neurodegeneration and prolongs survival in amyotrophic lateral sclerosis mice. J Neurosci 2007 Jan 10;27(2):304-307.

(239) Ascherio A, Weisskopf MG, O'Reilly E, Jacobs EJ, McCullough ML, Calle EE, et al. Vitamin E intake and risk of amyotrophic lateral sclerosis. Ann Neurol 2005;57(1):104-110.

(240) Turner MR, Wotton C, Talbot K, Goldacre MJ. Cardiovascular fitness as a risk factor for amyotrophic lateral sclerosis: indirect evidence from record linkage study. J Neurol Neurosurg Psychiatry 2012 Apr;83(4):395-398.

(241) Kim SM, Kim H, Kim JE, Park KS, Sung JJ, Kim SH, et al. Amyotrophic lateral sclerosis is associated with hypolipidemia at the presymptomatic stage in mice. PLoS One 2011 Mar 25;6(3):e17985.

(242) Paganoni S, Deng J, Jaffa M, Cudkowicz ME, Wills AM. What does body mass index measure in amyotrophic lateral sclerosis and why should we care? Muscle Nerve 2012 Apr;45(4):612.

(243) Dorst J, Kuhnlein P, Hendrich C, Kassubek J, Sperfeld AD, Ludolph AC. Patients with elevated triglyceride and cholesterol serum levels have a prolonged survival in amyotrophic lateral sclerosis. J Neurol 2011 Apr;258(4):613-617.

(244) Chio A, Borghero G, Pugliatti M, Ticca A, Calvo A, Moglia C, et al. Large proportion of amyotrophic lateral sclerosis cases in Sardinia due to a single founder mutation of the TARDBP gene. Arch Neurol 2011 May;68(5):594-598.

(245) Popat RA, Van Den Eeden SK, Tanner CM, Bernstein AL, Bloch DA, Leimpeter A, et al. Effect of reproductive factors and postmenopausal hormone use on the risk of amyotrophic lateral sclerosis. Neuroepidemiology 2006;27(3):117-121.

(246) Rita Ashok Popat. Reproductive history and pharmacological agents as risk factors for amyotrophic lateral sclerosis and Parkinson's disease. United States -- California: Stanford University; 2003.

(247) de Jong S, Huisman M, Sutedja N, van der Kooi A, de Visser M, Schelhaas J, et al. Endogenous female reproductive hormones and the risk of amyotrophic lateral sclerosis. J Neurol 2013 Feb;260(2):507-512.

(248) Freedman D, Travis LB, Gridley G, Kuncl RW. Amyotrophic Lateral Sclerosis Mortality in 1.9 Million US Cancer Survivors. Neuroepidemiology 2005;25(4):176-180.

(249) Freedman DM, Curtis RE, Daugherty SE, Goedert JJ, Kuncl RW, Tucker MA. The association between cancer and amyotrophic lateral sclerosis. Cancer Causes Control 2012 Oct 23.

(250) Mitchell JD, Davies RB, al-Hamad A, Gatrell AC, Batterby G. MND risk factors: an epidemiological study in the north west of England. J Neurol Sci 1995 May;129 Suppl:61-64.

(251) Dupuis L, Pradat PF, Ludolph AC, Loeffler JP. Energy metabolism in amyotrophic lateral sclerosis. Lancet Neurol 2011 Jan;10(1):75-82.

(252) Boeve BF, Graff-Radford NR. Cognitive and behavioral features of c9FTD/ALS. Alzheimers Res Ther 2012 Jul 20;4(4):29.

(253) Kaji R, Izumi Y, Adachi Y, Kuzuhara S. ALS-Parkinsonism-Dementia complex of Kii and other related diseases in Japan. Parkinsonism Relat Disord 2012 Jan;18 Suppl 1:S190-1.

(254) Fang F, Ye W, Fall K, Lekander M, Wigzell H, Sparén P, et al. Loss of a child and the risk of amyotrophic lateral sclerosis. Am J Epidemiol 2008 15;167(2):203-210.

(255) Plato C, Galasko D, Garruto R, Plato M, Gamst A, Craig U, et al. ALS and PDC of Guam - Forty-year follow-up. Neurology 2002;58(5):765-773.

(256) Cox P, Richer R, Metcalf J, Banack S, Codd G, Bradley W. Cyanobacteria and BMAA exposure from desert dust: A possible link to sporadic ALS among Gulf War veterans. Amyotrophic Lat Scler 2009;10:109-117.

(257) Karamyan VT, Speth RC. Animal models of BMAA neurotoxicity: A critical review. Life Sci 2008;82(5-6):233-246.

(258) Cox PA, Sacks OW. Cycad neurotoxins, consumption of flying foxes, and ALS-PDC disease in Guam. Neurology, March 26 2002;58(6):956-959.

(259) Cox P, Banack S, Murch S. Biomagnification of cyanobacterial neurotoxins and neurodegenerative disease among the Chamorro people of Guam. Proc Natl Acad Sci USA 2003 11 Nov;100(23):13380-13383.

(260) Spencer PS, Hugon J, Ludolph A, Nunn PB, Ross SM, Roy DN, et al. Discovery and partial characterization of primate motor-system toxins. Ciba Found Symp 1987;126:221-238.

(261) Spencer PS, Nunn PB, Hugon J, Ludolph AC, Ross SM, Roy DN, et al. Guam amyotrophic lateral sclerosis-parkinsonism-dementia linked to a plant excitant neurotoxin. Science 1987 Jul 31;237(4814):517-522.

(262) Spencer PS. Guam ALS/parkinsonism-dementia: a long-latency neurotoxic disorder caused by "slow toxin(s)" in food? Can J Neurol Sci 1987 Aug;14(3 Suppl):347-357.

(263) Banack SA, Caller TA, Stommel EW. The cyanobacteria derived toxin Beta-N-methylamino-L-alanine and amyotrophic lateral sclerosis. Toxins (Basel) 2010 Dec;2(12):2837-2850.

(264) Murch SJ, Cox PA, Banack SA, Steele JC, Sacks OW. Occurrence of beta-methylamino-l-alanine (BMAA) in ALS/PDC patients from Guam. Acta Neurol Scand 2004 Oct;110(4):267-269.

(265) Caller TA, Field NC, Chipman JW, Shi X, Harris BT, Stommel EW. Spatial clustering of amyotrophic lateral sclerosis and the potential role of BMAA. Amyotroph Lateral Scler 2012 Jan;13(1):25-32.

(266) Caller TA, Doolin JW, Haney JF, Murby AJ, West KG, Farrar HE, et al. A cluster of amyotrophic lateral sclerosis in New Hampshire: a possible role for toxic cyanobacteria blooms. Amyotrophic lateral sclerosis : official publication of the World Federation of Neurology Research Group on Motor Neuron Diseases, Jan 01 2009;10 Suppl 2:101-108.

(267) Masseret E, Banack S, Boumediene F, Abadie E, Brient L, Pernet F, et al. Dietary BMAA Exposure in an Amyotrophic Lateral Sclerosis Cluster from Southern France. PLoS One 2013 Dec 13;8(12):e83406.

(268) Field NC, Metcalf JS, Caller TA, Banack SA, Cox PA, Stommel EW. Linking beta-methylamino-L-alanine exposure to sporadic amyotrophic lateral sclerosis in Annapolis, MD. Toxicon 2013 Aug;70:179-183.

(269) de Munck E, Munoz-Saez E, Miguel BG, Solas MT, Ojeda I, Martinez A, et al. beta-N-methylamino-l-alanine causes neurological and pathological phenotypes mimicking Amyotrophic Lateral Sclerosis (ALS): the first step towards an experimental model for sporadic ALS. Environ Toxicol Pharmacol 2013 Sep;36(2):243-255.

(270) Haddock RL, Chen KM. Amyotrophic lateral sclerosis and diabetes on Guam: changing patterns of chronic disease in an island community. Southeast Asian J Trop Med Public Health 2003 Sep;34(3):659-661.

(271) Ahlskog JE, Petersen RC, Waring SC, Esteban-Santillan C, Craig UK, Maraganore DM, et al. Guamanian neurodegenerative disease: are diabetes mellitus and altered humoral immunity clues to pathogenesis? Neurology 1997 May;48(5):1356-1362.

(272) Al-Chalabi A, Lewis CM. Modelling the effects of penetrance and family size on rates of sporadic and familial disease. Hum Hered 2011;71(4):281-288.

(273) Jones CT, Brock DJ, Chancellor AM, Warlow CP, Swingler RJ. Cu/Zn superoxide dismutase (SOD1) mutations and sporadic amyotrophic lateral sclerosis. Lancet 1993 Oct 23;342(8878):1050-1051.

(274) Ripps ME, Huntley GW, Hof PR, Morrison JH, Gordon JW. Transgenic mice expressing an altered murine superoxide dismutase gene provide an animal model of amyotrophic lateral sclerosis. Proc Natl Acad Sci U S A 1995 Jan 31;92(3):689-693.

(275) PRIZE4LIFE. ALSgene. Available at: http://www.alsgene.org/. Accessed January/25, 2014.

(276) Renton AE, Majounie E, Waite A, Simon-Sanchez J, Rollinson S, Gibbs JR, et al. A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. Neuron 2011 Oct 20;72(2):257-268.

(277) Dejesus-Hernandez M, Rayaprolu S, Soto-Ortolaza AI, Rutherford NJ, Heckman MG, Traynor S, et al. Analysis of the C9orf72 repeat in Parkinson's disease, essential tremor and restless legs syndrome. Parkinsonism Relat Disord 2012 Oct 17.

(278) Garcia-Redondo A, Dols-Icardo O, Rojas-Garcia R, Esteban-Perez J, Cordero-Vazquez P, Munoz-Blanco JL, et al. Analysis of the C9orf72 gene in patients with amyotrophic lateral sclerosis in Spain and different populations worldwide. Hum Mutat 2013 Jan;34(1):79-82.

(279) Zou ZY, Li XG, Liu MS, Cui LY. Screening for C9orf72 repeat expansions in Chinese amyotrophic lateral sclerosis patients. Neurobiol Aging 2013 Jun;34(6):1710.e5-1710.e6.

(280) Alavi A, Nafissi S, Rohani M, Shahidi G, Zamani B, Shamshiri H, et al. Repeat expansion in C9ORF72 is not a major cause of amyotrophic lateral sclerosis among Iranian patients. Neurobiol Aging 2013 Aug 17.

(281) Elden AC, Kim H-, Hart MP, Chen-Plotkin AS, Johnson BS, Fang X, et al. Ataxin-2 intermediate-length polyglutamine expansions are associated with increased risk for ALS. Nature 2010 26 Aug 2010;466(7310):1069-1075.

(282) Downs SH, Black N. The feasibility of creating a checklist for the assessment of the methodological quality both of randomised and non-randomised studies of health care interventions. J Epidemiol Community Health 1998 Jun;52(6):377-384.

(283) Laffita-Mesa JM, Rodriguez Pupo JM, Moreno Sera R, Vazquez Mojena Y, Kouri V, Laguna-Salvia L, et al. De Novo Mutations in Ataxin-2 Gene and ALS Risk. PLoS One 2013 Aug 6;8(8):e70560.

(284) Corrado L, Mazzini L, Oggioni GD, Luciano B, Godi M, Brusco A, et al. ATXN-2 CAG repeat expansions are interrupted in ALS patients. Hum Genet 2011 October 2011;130(4):575-580.

(285) Conforti FL, Spataro R, Sproviero W, Mazzei R, Cavalcanti F, Condino F, et al. Ataxin-1 and ataxin-2 intermediate-length PolyQ expansions in amyotrophic lateral sclerosis. Neurology Dec 2012;79(24):2315-2320.

(286) Bonini NM, Gitler AD. Model organisms reveal insight into human neurodegenerative disease: ataxin-2 intermediate-length polyglutamine expansions are a risk factor for ALS. J Mol Neurosci 2011 Nov;45(3):676-683.

(287) Ross OA, Rutherford NJ, Baker M, Soto-Ortolaza AI, Carrasquillo MM, DeJesus-Hernandez M, et al. Ataxin-2 repeat-length variation and neurodegeneration. Hum Mol Genet 2011 Aug 15;20(16):3207-3212.

(288) Van Langenhove T, van der Zee J, Engelborghs S, Vandenberghe R, Santens P, Van den Broeck M, et al. Ataxin-2 polyQ expansions in FTLD-ALS spectrum disorders in Flanders-Belgian cohorts. Neurobiol Aging May 2012;33(5):e17-e20.

(289) Gellera C, Ticozzi N, Pensato V, Nanetti L, Castucci A, Castellotti B, et al. ATAXIN2 CAG-repeat length in Italian patients with amyotrophic lateral sclerosis: Risk factor or variant phenotype? Implication for genetic testing and counseling. Neurobiol Aging Aug 2012;33(8):e15-e21.

(290) Lee T, Li YR, Chesi A, Hart MP, Ramos D, Jethava N, et al. Evaluating the prevalence of polyglutamine repeat expansions in amyotrophic lateral sclerosis. Neurology Jun 2011;76(24):2062-2065.

(291) Daoud H, Belzil V, Martins S, Sabbagh M, Provencher P, Lacomblez L, et al. Association of long ATXN2 CAG repeat sizes with increased risk of amyotrophic lateral sclerosis. Arch Neurol Jun 2011;68(6):739-742.

(292) Van Damme P, Veldink JH, van Blitterswijk M, Corveleyn A, van Vught, P. W. J, Thijs V, et al. Expanded ATXN2 CAG repeat size in ALS identifies genetic overlap between ALS and SCA2. Neurology Jun 2011;76(24):2066-2072.

(293) Lee T, Li YR, Ingre C, Weber M, Grehl T, Gredal O, et al. Ataxin-2 intermediate-length polyglutamine expansions in European ALS patients. Hum Mol Genet 2011 May 1;20(9):1697-1700.

(294) Gispert S, Kurz A, Waibel S, Bauer P, Liepelt I, Geisen C, et al. The modulation of Amyotrophic Lateral Sclerosis risk by Ataxin-2 intermediate polyglutamine expansions is a specific effect. Neurobiol Dis 2012 January 2012;45(1):356-361.

(295) Liu X, Lu M, Tang L, Zhang N, Chui D, Fan D. ATXN2 CAG repeat expansions increase the risk for Chinese patients with amyotrophic lateral sclerosis. Neurobiol Aging Sep 2013;34(9):e5-e8.

(296) Chen Y, Huang R, Yang Y, Chen K, Song W, Pan P, et al. Ataxin-2 intermediate-length polyglutamine: A possible risk factor for Chinese patients with amyotrophic lateral sclerosis. Neurobiol Aging Oct 2011;32(10):e1-e5.

(297) Laffita J, Bauer PO, Kouri V, Pena Serrano L, Roskams J, Almaguer Gotay D, et al. Epigenetic DNA-methylation in the core ataxin-2 gene promoter: Novel physiological and pathological implications. Parkinsonism and Related Disorders 2012 January 2012;18:S187.

(298) Lahut S, Omur O, Uyan O, Agim ZS, Ozoguz A, Parman Y, et al. ATXN2 and its neighbouring gene SH2B3 are associated with increased ALS risk in the Turkish population. PLoS One 2012;7(8):e42956.

(299) Yu Z, Zhu Y, Chen-Plotkin AS, Clay-Falcone D, McCluskey L, Elman L, et al. PolyQ repeat expansions in ATXN2 associated with ALS are CAA interrupted repeats. PLoS One 2011 Mar 29;6(3):e17951.

(300) Hart MP, Brettschneider J, Lee VMY, Trojanowski JQ, Gitler AD. Distinct TDP-43 pathology in ALS patients with ataxin 2 intermediate-length polyQ expansions. Acta Neuropathol Aug 2012;124(2):221-230.

(301) Hart MP, Gitler AD. ALS-associated ataxin 2 polyQ expansions enhance stress-induced caspase 3 activation and increase TDP-43 pathological modifications. The Journal of Neuroscience Jul 2012;32(27):9133-9142.

(302) Drost J, Nonis D, Eich F, Leske O, Damrath E, Brunt ER, et al. Ataxin-2 Modulates the Levels of Grb2 and Src but Not Ras Signaling. J Mol Neurosci 2013 Jan 19.

(303) Gellera C, Ticozzi N, Pensato V, Nanetti L, Castucci A, Castellotti B, et al. ATAXIN2 CAG-repeat length in Italian patients with amyotrophic lateral sclerosis: risk factor or variant phenotype? Implication for genetic testing and counseling. Neurobiol Aging 2012 Mar 15.

(304) Gispert S, Kurz A, Waibel S, Bauer P, Liepelt I, Geisen C, et al. The modulation of Amyotrophic Lateral Sclerosis risk by ataxin-2 intermediate polyglutamine expansions is a specific effect. Neurobiol Dis 2012 Jan;45(1):356-361.

(305) Chen X-, Sun H, Zhang C-, Zhang Y, Lin K-, Yu L, et al. Positive selection of CAG repeats of the ATXN2 gene in Chinese ethnic groups. Journal of Genetics and Genomics 2013 20 Oct 2013;40(10):543-548.

(306) Geschwind DH, Perlman S, Figueroa CP, Treiman LJ, Pulst SM. The prevalence and wide clinical spectrum of the spinocerebellar ataxia type 2 trinucleotide repeat in patients with autosomal dominant cerebellar ataxia. Am J Hum Genet 1997 Apr;60(4):842-850.

(307) Soraru G, Clementi , M, Forzan , M, Orsetti V, D'Ascenzo C, Querin G, et al. ALS risk but not phenotype is affected by ataxin-2 intermediate length polyglutamine expansion. Neurology Jun 2011;76(23):2030-2031.

(308) Laffita-Mesa JM, Velazquez-Perez LC, Santos Falcon N, Cruz-Marino T, Gonzalez Zaldivar Y, Vazquez Mojena Y, et al. Unexpanded and intermediate CAG polymorphisms at the SCA2 locus (ATXN2) in the Cuban population: evidence about the origin of expanded SCA2 alleles. Eur J Hum Genet 2012 Jan;20(1):41-49.

(309) Costanzi-Porrini S, Tessarolo D, Abbruzzese C, Liguori M, Ashizawa T, Giacanelli M. An interrupted 34-CAG repeat SCA-2 allele in patients with sporadic spinocerebellar ataxia. Neurology 2000 Jan 25;54(2):491-493.

(310) Fernandez M, McClain ME, Martinez RA, Snow K, Lipe H, Ravits J, et al. Late-onset SCA2: 33 CAG repeats are sufficient to cause disease. Neurology 2000 Aug 22;55(4):569-572.

(311) Tazen S, Figueroa K, Kwan JY, Goldman J, Hunt A, Sampson J, et al. Amyotrophic lateral sclerosis and spinocerebellar ataxia type 2 in a family with full CAG repeat expansions of ATXN2. Movement Disorders 2013 June 2013;28:S241-S242.

(312) Gispert S, Twells R, Orozco G, Brice A, Weber J, Heredero L, et al. Chromosomal assignment of the second locus for autosomal dominant cerebellar ataxia (SCA2) to chromosome 12q23-24.1. Nat Genet 1993 Jul;4(3):295-299.

(313) Magana JJ, Velazquez-Perez L, Cisneros B. Spinocerebellar ataxia type 2: clinical presentation, molecular mechanisms, and therapeutic perspectives. Mol Neurobiol 2013 Feb;47(1):90-104.

(314) Farg MA, Soo KY, Warraich ST, Sundaramoorthy V, Blair IP, Atkin JD. Ataxin-2 interacts with FUS and intermediate-length polyglutamine expansions enhance FUS-related pathology in amyotrophic lateral sclerosis. Hum Mol Genet 2013 Feb 15;22(4):717-728.

(315) Lattante S, Rouleau GA, Kabashi E. TARDBP and FUS mutations associated with amyotrophic lateral sclerosis: summary and update. Hum Mutat 2013 Jun;34(6):812-826.

(316) Andersen PM, Forsgren L, Binzer M, Nilsson P, Ala-Hurula V, Keranen ML, et al. Autosomal recessive adult-onset amyotrophic lateral sclerosis associated with homozygosity for Asp90Ala CuZn-superoxide dismutase mutation. A clinical and genealogical study of 36 patients. Brain 1996 Aug;119 (Pt 4)(Pt 4):1153-1172.

(317) Choudhry S, Mukerji M, Srivastava AK, Jain S, Brahmachari SK. CAG repeat instability at SCA2 locus: anchoring CAA interruptions and linked single nucleotide polymorphisms. Hum Mol Genet 2001 Oct 1;10(21):2437-2446.

(318) Ramos EM, Keagle P, Gillis T, Lowe P, Mysore JS, Leclerc AL, et al. Prevalence of Huntington's disease gene CAG repeat alleles in sporadic amyotrophic lateral sclerosis patients. Amyotroph Lateral Scler 2012 Mar 13.

(319) Ramos EM, Martins S, Alonso I, Emmel VE, Saraiva-Pereira ML, Jardim LB, et al. Common origin of pure and interrupted repeat expansions in spinocerebellar ataxia type 2 (SCA2). Am J Med Genet B Neuropsychiatr Genet 2010 Mar 5;153B(2):524-531.

(320) BragaNeto P, Pedroso JL, Felicio AC, Abrahao A, Dutra LA, Escorcio Bezerra ML, et al. SCA2 presenting as an ataxia-Parkinsonism-motor neuron disease syndrome. Arq Neuropsiquiatr Apr 2011;69((2-B):405-406.

(321) Qureshi AI, Wilmot G, Dihenia B, Schneider JA, Krendel DA. Motor neuron disease with parkinsonism. Arch Neurol 1996 Oct;53(10):987-991.

(322) Lee T, Li YR, Ingre C, Weber M, Grehl T, Gredal O, et al. Ataxin-2 intermediate-length polyglutamine expansions in European ALS patients. Hum Mol Genet 2011;20(9):1697-1700.

(323) Vinceti M, Fiore M, Signorelli C, Odone A, Tesauro M, Consonni M, et al. Environmental risk factors for amyotrophic lateral sclerosis: methodological issues in epidemiologic studies. Ann Ig 2012 Sep-Oct;24(5):407-415.

(324) Oh SS, Kim EA, Lee SW, Kim MK, Kang SK. A case of amyotrophic lateral sclerosis in electronic parts manufacturing worker exposed to lead. Neurotoxicology 2007;28(2):324-327.

(325) Livesley B, Sissons CE. Chronic lead intoxication mimicking motor neurone disease. Br Med J 1968 Nov 9;4(5627):387-388.

(326) Campbell AM, Williams ER, Barltrop D. Motor neurone disease and exposure to lead. J Neurol Neurosurg Psychiatry 1970 Dec;33(6):877-885.

(327) Deapen DM, Henderson BE. A case-control study of amyotrophic lateral sclerosis. Am J Epidemiol 1986 May;123(5):790-799.

(328) Dennis Martyn Deapen. A CASE-CONTROL STUDY OF AMYOTROPHIC LATERAL SCLEROSIS. United States -- California: University of California, Los Angeles; 1982.

(329) Gresham LS, Molgaard CA, Golbeck AL, Smith R. Amyotrophic lateral sclerosis and occupational heavy metal exposure: a case-control study. Neuroepidemiology 1986;5(1):29-38.

(330) Gresham LS, Molgaard CA, Golbeck AL, Smith R. Amyotrophic lateral sclerosis and history of skeletal fracture: a case-control study. Neurology 1987 Apr;37(4):717-719.

(331) Gresham LS, Molgaard CA, Golbeck AL, Smith R. Lead exposure and ALS. Neurology 1992 Nov;42(11):2228-2229.

(332) Kamel F, Umbach DM, Hu H, Munsat TL, Shefner JM, Taylor JA, et al. Lead exposure as a risk factor for amyotrophic lateral sclerosis. Neurodegener Dis 2005;2(3-4):195-201.

(333) Binazzi A, Belli S, Uccelli R, Desiato MT, Talamanca IF, Antonini G, et al. An exploratory case-control study on spinal and bulbar forms of amyotrophic lateral sclerosis in the province of Rome. Amyotroph Lateral Scler 2009 Oct-Dec;10(5-6):361-369.

(334) Provinciali L, Giovagnoli AR. Antecedent events in amyotrophic lateral sclerosis: do they influence clinical onset and progression? Neuroepidemiology 1990;9(5):255-262.

(335) Feychting M, Jonsson F, Pedersen NL, Ahlbom A. Occupational magnetic field exposure and neurodegenerative disease. Epidemiology 2003 Jul;14(4):413-9; discussion 427-8.

(336) Scarpa M, Colombo A, Panzetti P, Sorgato P. Epidemiology of amyotrophic lateral sclerosis in the province of Modena, Italy. Influence of environmental exposure to lead. Acta Neurol Scand 1988;77(6):456-460.

(337) Turabelidze G, Zhu BP, Schootman M, Malone JL, Horowitz S, Weidinger J, et al. An epidemiologic investigation of amyotrophic lateral sclerosis in Jefferson County, Missouri, 1998-2002. Neurotoxicology 2008;29(1):81-86.

(338) Breslow N, Day N editors. Satatistical methods in cancer research. 1st ed. Lyon: IARC Scientific Publications; 1980.

(339) Valerie McGuire. Assessment of occupational exposures: Methodologic issues in a risk factor study of amyotrophic lateral sclerosis. United States -- Washington: University of Washington; 1996.

(340) Thomson RM, Parry GJ. Neuropathies associated with excessive exposure to lead. Muscle Nerve 2006 Jun;33(6):732-741.

(341) Bachmeyer C, Bagur E, Lenglet T, Maier-Redelsperger M, Lecomte I. Lead poisoning mimicking amyotrophic lateral sclerosis: an adverse effect of rituals. Am J Med 2012 Jun;125(6):e5-6.

(342) Fluri F, Lyrer P, Gratwohl A, Raetz-Bravo AE, Steck AJ. Lead poisoning from the beauty case: neurologic manifestations in an elderly woman. Neurology 2007 Aug 28;69(9):929-930.

(343) Boothby JA, DeJesus PV, Rowland LP. Reversible forms of motor neuron disease. Lead "neuritis". Arch Neurol 1974 Jul;31(1):18-23.

(344) Felmus MT, Patten BM, Swanke L. Antecedent events in amyotrophic lateral sclerosis. Neurology 1976 Feb;26(2):167-172.

(345) Mandybur TI, Cooper GP. Increased spinal cord lead content in amyotrophic lateral sclerosis--possibly a secondary phenomenon. Med Hypotheses 1979 Dec;5(12):1313-1315.

(346) Petkau A, Sawatzky A, Hillier CR, Hoogstraten J. Lead content of neuromuscular tissue in amyotrophic lateral sclerosis: case report and other considerations. Br J Ind Med 1974 Oct;31(4):275-287.

(347) Conradi S, Ronnevi LO, Vesterberg O. Abnormal tissue distribution of lead in amyotrophic lateral sclerosis. J Neurol Sci 1976 Oct;29(2-4):259-265.

(348) Conradi S, Ronnevi LO, Vesterberg O. Increased plasma levels of lead in patients with amyotrophic lateral sclerosis compared with control subjects as determined by flameless atomic absorption spectrophotometry. J Neurol Neurosurg Psychiatry 1978 May;41(5):389-393.

(349) Roos PM, Vesterberg O, Syversen T, Flaten TP, Nordberg M. Metal concentrations in cerebrospinal fluid and blood plasma from patients with amyotrophic lateral sclerosis. Biol Trace Elem Res 2013 Feb;151(2):159-170.

(350) Conradi S, Ronnevi LO, Vesterberg O. Lead concentration in skeletal muscle in amyotrophic lateral sclerosis patients and control subjects. J Neurol Neurosurg Psychiatry 1978 Nov;41(11):1001-1004.

(351) Vinceti M, Guidetti D, Bergomi M, Caselgrandi E, Vivoli R, Olmi M, et al. Lead, cadmium, and selenium in the blood of patients with sporadic amyotrophic lateral sclerosis. Ital J Neurol Sci 1997 Apr;18(2):87-92.

(352) Kapaki E, Segditsa J, Zournas C, Xenos D, Papageorgiou C. Determination of cerebrospinal fluid and serum lead levels in patients with amyotrophic lateral sclerosis and other neurological diseases. Experientia 1989 Dec 1;45(11-12):1108-1110.

(353) Cavalleri A, Minoia C, Ceroni M, Poloni M. Lead in cerebrospinal fluid and its relationship to plasma lead in humans. J Appl Toxicol 1984 Apr;4(2):63-65.

(354) Stober T, Stelte W, Kunze K. Lead concentrations in blood, plasma, erythrocytes, and cerebrospinal fluid in amyotrophic lateral sclerosis. J Neurol Sci 1983 Sep;61(1):21-26.

(355) Conradi S, Ronnevi LO, Nise G, Vesterberg O. Abnormal distribution of lead in amyotrophic lateral sclerosis--reestimation of lead in the cerebrospinal fluid. J Neurol Sci 1980 Dec;48(3):413-418.

(356) Damstra T. Toxicological properties of lead. Environ Health Perspect 1977 Aug;19:297-307.

(357) Mazliah J, Barron S, Bental E, Rogowski Z, Coleman R, Silbermann M. The effects of long-term lead intoxication on the nervous system of the chicken. Neurosci Lett 1989 Jul 3;101(3):253-257.

(358) Mulligan VK, Chakrabartty A. Protein misfolding in the late-onset neurodegenerative diseases: common themes and the unique case of amyotrophic lateral sclerosis. Proteins 2013 Aug;81(8):1285-1303.

(359) Kim S, Hyun J, Kim H, Kim Y, Kim E, Jang J, et al. Effects of lead exposure on nitric oxide-associated gene expression in the olfactory bulb of mice. Biol Trace Elem Res 2011 Sep;142(3):683-692.

(360) Baranowska-Bosiacka I, Gutowska I, Marchlewicz M, Marchetti C, Kurzawski M, Dziedziejko V, et al. Disrupted pro- and antioxidative balance as a mechanism of neurotoxicity induced by perinatal exposure to lead. Brain Res 2012 Jan 30;1435:56-71.

(361) Rotunno MS, Bosco DA. An emerging role for misfolded wild-type SOD1 in sporadic ALS pathogenesis. Front Cell Neurosci 2013 Dec 16;7:253.

(362) Goering PL. Lead-protein interactions as a basis for lead toxicity. Neurotoxicology 1993 Summer-Fall;14(2-3):45-60.

(363) Grad LI, Cashman NR. Prion-like activity of Cu/Zn superoxide dismutase: Implications for amyotrophic lateral sclerosis. Prion 2014 Jan 1;8(1).

(364) Grad LI, Guest WC, Yanai A, Pokrishevsky E, O'Neill MA, Gibbs E, et al. Intermolecular transmission of superoxide dismutase 1 misfolding in living cells. Proc Natl Acad Sci U S A 2011 Sep 27;108(39):16398-16403.

(365) Rosin A. The long-term consequences of exposure to lead. Isr Med Assoc J 2009 Nov;11(11):689-694.

(366) Armon C, Kurland LT, O'Brien PC, Mulder DW. Antecedent medical diseases in patients with amyotrophic lateral sclerosis. A population-based case-controlled study in Rochester, Minn, 1925 through 1987. Arch Neurol 1991 Mar;48(3):283-286.

(367) Chancellor AM, Hendry A, Caird FI, Warlow CP, Weir AI. Motor neuron disease: a disease of old age. Scott Med J 1993 Dec;38(6):178-182.

(368) Roelofs-Iverson RA, Mulder DW, Elveback LR, Kurland LT, Molgaard CA. ALS and heavy metals: a pilot case-control study. Neurology 1984 Mar;34(3):393-395.

(369) Sato K, Morimoto N, Deguchi K, Ikeda Y, Matsuura T, Abe K. Seven amyotrophic lateral sclerosis patients diagnosed only after development of respiratory failure. J Clin Neurosci 2014 Feb 6.

(370) Yamashita S, Mori A, Sakaguchi H, Suga T, Ishihara D, Ueda A, et al. Sporadic juvenile amyotrophic lateral sclerosis caused by mutant FUS/TLS: possible association of mental retardation with this mutation. J Neurol 2011 Nov 5.

(371) Belzil VV, Langlais JS, Daoud H, Dion PA, Brais B, Rouleau GA. Novel FUS Deletion in a Patient With Juvenile Amyotrophic Lateral Sclerosis. Arch Neurol 2012 Jan 16.

(372) Al-Saif A, Al-Mohanna F, Bohlega S. A mutation in sigma-1 receptor causes juvenile amyotrophic lateral sclerosis. Ann Neurol 2011 Aug 12.

(373) Avemaria F, Lunetta C, Tarlarini C, Mosca L, Maestri E, Marocchi A, et al. Mutation in the senataxin gene found in a patient affected by familial ALS with juvenile onset and slow progression. Amyotroph Lateral Scler 2011 May;12(3):228-230.

(374) Deng HX, Chen W, Hong ST, Boycott KM, Gorrie GH, Siddique N, et al. Mutations in UBQLN2 cause dominant X-linked juvenile and adult-onset ALS and ALS/dementia. Nature 2011 Aug 21;477(7363):211-215.

(375) Miller RG, Mitchell JD, Moore DH. Riluzole for amyotrophic lateral sclerosis (ALS)/motor neuron disease (MND). Cochrane Database Syst Rev 2012 Mar 14;3:CD001447.

(376) Goh KJ, Tian S, Shahrizaila N, Ng CW, Tan CT. Survival and prognostic factors of motor neuron disease in a multi-ethnic Asian population. Amyotroph Lateral Scler 2011 Mar;12(2):124-129.

(377) Costa J, Swash M, de Carvalho M. Awaji criteria for the diagnosis of amyotrophic lateral sclerosis:a systematic review. Arch Neurol 2012 Nov;69(11):1410-1416.

(378) Vender RL, Mauger D, Walsh S, Alam S, Simmons Z. Respiratory systems abnormalities and clinical milestones for patients with amyotrophic lateral sclerosis with emphasis upon survival. Amyotroph Lateral Scler 2007 Feb;8(1):36-41.

(379) Yang R, Huang R, Chen D, Song W, Zeng Y, Zhao B, et al. Causes and places of death of patients with amyotrophic lateral sclerosis in south-west China. Amyotroph Lateral Scler 2011 May;12(3):206-209.

(380) Spataro R, Lo Re M, Piccoli T, Piccoli F, La Bella V. Causes and place of death in Italian patients with amyotrophic lateral sclerosis. Acta Neurol Scand 2010 Sep;122(3):217-223.

(381) Chio A, Logroscino G, Hardiman O, Swingler R, Mitchell D, Beghi E, et al. Prognostic factors in ALS: A critical review. Amyotroph Lateral Scler 2009 Oct-Dec;10(5-6):310-323.

(382) Mateen FJ, Carone M, Sorenson EJ. Patients who survive 5 years or more with ALS in Olmsted County, 1925-2004. J Neurol Neurosurg Psychiatry 2010 Oct;81(10):1144-1146.

(383) Logroscino G, Traynor BJ, Hardiman O, Chio' A, Couratier P, Mitchell JD, et al. Descriptive epidemiology of amyotrophic lateral sclerosis: new evidence and unsolved issues. J Neurol Neurosurg Psychiatry 2008 Jan;79(1):6-11.

(384) Byrne S, Bede P, Elamin M, Kenna K, Lynch C, McLaughlin R, et al. Proposed criteria for familial amyotrophic lateral sclerosis. Amyotroph Lateral Scler 2011 May;12(3):157-159.

(385) Cannon JR, Timothy Greenamyre J. The role of environmental exposures in neurodegeneration and neurodegenerative diseases. Toxicol Sci 2011 Sep 13.

(386) Burns CJ, Beard KK, Cartmill JB. Mortality in chemical workers potentially exposed to 2,4-dichlorophenoxyacetic acid (2,4-D) 1945-94: an update. Occup Environ Med 2001 Jan;58(1):24-30.

(387) Pall HS, Williams AC, Waring R, Elias E. Motoneurone disease as manifestation of pesticide toxicity. Lancet 1987 Sep 19;2(8560):685.

(388) Fonseca RG, Resende LA, Silva MD, Camargo A. Chronic motor neuron disease possibly related to intoxication with organochlorine insecticides. Acta Neurol Scand 1993 Jul;88(1):56-58.

(389) Ahdab R, Ayache SS, Maltonti F, Brugieres P, Lefaucheur JP. Motor neuron disorder with tongue spasms due to pyrethroid insecticide toxicity. Neurology 2011 Jan 11;76(2):196-197.

(390) Kanavouras K, Tzatzarakis MN, Mastorodemos V, Plaitakis A, Tsatsakis AM. A case report of motor neuron disease in a patient showing significant level of DDTs, HCHs and organophosphate metabolites in hair as well as levels of hexane and toluene in blood. Toxicol Appl Pharmacol 2011 Nov 1;256(3):399-404.

(391) Doi H, Kikuchi H, Murai H, Kawano Y, Shigeto H, Ohyagi Y, et al. Motor neuron disorder simulating ALS induced by chronic inhalation of pyrethroid insecticides. Neurology 2006 Nov 28;67(10):1894-1895.

(392) Das K, Nag C, Ghosh M. Familial, environmental, and occupational risk factors in development of amyotrophic lateral sclerosis. N Am J Med Sci 2012 Aug;4(8):350-355.

(393) Kondo K, Tsubaki T. Case-control studies of motor neuron disease: association with mechanical injuries. Arch Neurol 1981 Apr;38(4):220-226.

(394) Migliaretti G, Berchialla P, Dalmasso P, Cavallo F, Chio A. Amyotrophic lateral sclerosis in Piedmont (Italy): a Bayesian spatial analysis of the incident cases. Amyotroph Lateral Scler Frontotemporal Degener 2013 Jan;14(1):58-65.

(395) Lee CT, Chiu YW, Wang KC, Hwang CS, Lin KH, Lee IT, et al. Riluzole and prognostic factors in amyotrophic lateral sclerosis long-term and short-term survival: a population-based study of 1149 cases in Taiwan. J Epidemiol 2013 Jan 5;23(1):35-40.

(396) Bettoni L, Bazzani M, Bortone E, Dascola I, Pisani E, Mancia D. Steadiness of amyotrophic lateral sclerosis in the province of Parma, Italy, 1960-1990. Acta Neurol Scand 1994 Oct;90(4):276-280.

(397) Jokanovic M, Kosanovic M. Neurotoxic effects in patients poisoned with organophosphorus pesticides. Environ Toxicol Pharmacol 2010 May;29(3):195-201.

(398) McKhann GM, Cornblath DR, Griffin JW, Ho TW, Li CY, Jiang Z, et al. Acute motor axonal neuropathy: a frequent cause of acute flaccid paralysis in China. Ann Neurol 1993 Apr;33(4):333-342.

(399) Zhang C. The report of organophosphorus pesticides cause delayed nervous system diseases (143 cases). Zhonghua Shen Jing Jing Shen Ke Za Zhi 1991 Dec;24(6):336-8, 383.

(400) Barth S, Kang H, Bullman T, Wallin M. Neurological Mortality Among US Veterans of the Persian Gulf War: 13-Year Follow-Up. Am J Ind Med 2009;52(9):663-670.

(401) Horner RD, Kamins KG, Feussner JR, Grambow SC, Hoff-Lindquist J, Harati Y, et al. Occurrence of amyotrophic lateral sclerosis among Gulf War veterans. Neurology 2003 Sep 23;61(6):742-749.

(402) Haley RW, Billecke S, La Du BN. Association of low PON1 type Q (type A) arylesterase activity with neurologic symptom complexes in Gulf War veterans. Toxicol Appl Pharmacol 1999 Jun 15;157(3):227-233.

(403) Haley RW, Kurt TL. Self-reported exposure to neurotoxic chemical combinations in the Gulf War. A cross-sectional epidemiologic study. JAMA 1997 Jan 15;277(3):231-237.

(404) Mrema EJ, Rubino FM, Brambilla G, Moretto A, Tsatsakis AM, Colosio C. Persistent organochlorinated pesticides and mechanisms of their toxicity. Toxicology 2013 May 10;307:74-88.

(405) Mostafalou S, Abdollahi M. Pesticides and human chronic diseases: evidences, mechanisms, and perspectives. Toxicol Appl Pharmacol 2013 Apr 15;268(2):157-177.

(406) Rowland LP. How amyotrophic lateral sclerosis got its name: the clinical-pathologic genius of Jean-Martin Charcot. Arch Neurol 2001 Mar;58(3):512-515.

(407) McKee AC, Gavett BE, Stern RA, Nowinski CJ, Cantu RC, Kowall NW, et al. TDP-43 proteinopathy and motor neuron disease in chronic traumatic encephalopathy. J Neuropathol Exp Neurol 2010 Sep;69(9):918-929.

(408) Lehman EJ, Hein MJ, Baron SL, Gersic CM. Neurodegenerative causes of death among retired National Football League players. Neurology 2012 Nov 6;79(19):1970-1974.

(409) Riggs JE. Amyotrophic lateral sclerosis, heterogeneous susceptibility, trauma, and epidemiology. Arch Neurol 1996 Mar;53(3):225-227.

(410) Riggs JE. Antecedent trauma and amyotrophic lateral sclerosis in young adult men. Mil Med 1993 Jan;158(1):55-57.

(411) Riggs JE. The latency between traumatic axonal injury and the onset of amyotrophic lateral sclerosis in young adult men. MILIT MED 2001 08;166(8):731-732.

(412) Riggs JE, Hobbs GR. Motor axonal injury and amyotrophic lateral sclerosis: risk assessment using a reverse probability analysis technique. Mil Med 2003 Feb;168(2):143-145.

(413) Armon C, Nelson LM. Is head trauma a risk factor for amyotrophic lateral sclerosis? An evidence based review. Amyotroph Lateral Scler 2012 Mar 16.

(414) Bracco L, Antuono P, Amaducci L. Study of epidemiological and etiological factors of amyotrophic lateral sclerosis in the province of Florence, Italy. Acta Neurol Scand 1979 Aug;60(2):112-124.

(415) Gallagher JP, Sanders M. Trauma and amyotrophic lateral sclerosis: a report of 78 patients. Acta Neurol Scand 1987 Feb;75(2):145-150.

(416) Gawel M, Zaiwalla Z, Rose FC. Antecedent events in motor neuron disease. J Neurol Neurosurg Psychiatry 1983 Nov;46(11):1041-1043.

(417) Murros K, Fogelholm R. Amyotrophic lateral sclerosis in Middle-Finland: an epidemiological study. Acta Neurol Scand 1983 Jan;67(1):41-47.

(418) Pupillo E, Messina P, Logroscino G, Zoccolella S, Chio A, Calvo A, et al. Trauma and amyotrophic lateral sclerosis: a case-control study from a population-based registry. Eur J Neurol 2012 Dec;19(12):1509-1517.

(419) Strickland D, Smith SA, Dolliff G, Goldman L, Roelofs RI. Physical activity, trauma, and ALS: a case-control study. Acta Neurol Scand 1996 Jul;94(1):45-50.

(420) Kihira T, Kanno S, Miwa H, Okamoto K, Kondo T. The role of exogenous risk factors in amyotrophic lateral sclerosis in Wakayama, Japan. Amyotroph Lateral Scler 2007 Jun;8(3):150-156.

(421) Karttunen JP, Rautiainen RH. Distribution and characteristics of occupational injuries and diseases among farmers: a retrospective analysis of workers' compensation claims. Am J Ind Med 2013 Aug;56(8):856-869.

(422) Imaizumi Y. Mortality rate of amyotrophic lateral sclerosis in Japan: effects of marital status and social class, and geographical variation. Jinrui Idengaku Zasshi 1986 Jun;31(2):101-111.

(423) Valdés EG, Garbuzova-Davisb S. **Brain and Spinal Cord Trauma as a Risk Factor for Amyotrophic Lateral Sclerosis: A Mini-Review** . Open Journal of Neuroscience 2013;3-4.

(424) Peters TL, Fang F, Weibull CE, Sandler DP, Kamel F, Ye W. Severe head injury and amyotrophic lateral sclerosis. Amyotroph Lateral Scler Frontotemporal Degener 2013 May;14(4):267-272.

(425) Piazza O, Siren AL, Ehrenreich H. Soccer, neurotrauma and amyotrophic lateral sclerosis: is there a connection? Curr Med Res Opin 2004 Apr;20(4):505-508.

(426) Huisman MH, Seelen M, de Jong SW, Dorresteijn KR, van Doormaal PT, van der Kooi AJ, et al. Lifetime physical activity and the risk of amyotrophic lateral sclerosis. J Neurol Neurosurg Psychiatry 2013 Feb 16.

(427) Won J, Ahn Y, Song J, Koh D, Roh J. Occupational injuries in Korea: a comparison of blue-collar and white-collar workers' rates and underreporting. J Occup Health 2007 Jan;49(1):53-60.

(428) LeLorier J, Gregoire G, Benhaddad A, Lapierre J, Derderian F. Discrepancies between meta-analyses and subsequent large randomized, controlled trials. N Engl J Med 1997 Aug 21;337(8):536-542.

(429) Egger M, Smith GD, Phillips AN. Meta-analysis: principles and procedures. BMJ 1997 Dec 6;315(7121):1533-1537.

(430) Wartenberg D. Residential EMF exposure and childhood leukemia: meta-analysis and population attributable risk. Bioelectromagnetics 2001;Suppl 5:S86-104.

(431) Been JV, Lugtenberg MJ, Smets E, van Schayck CP, Kramer BW, Mommers M, et al. Preterm birth and childhood wheezing disorders: a systematic review and meta-analysis. PLoS Med 2014 Jan 28;11(1):e1001596.

(432) Tu JV, Nardi L, Fang J, Liu J, Khalid L, Johansen H, et al. National trends in rates of death and hospital admissions related to acute myocardial infarction, heart failure and stroke, 1994-2004. CMAJ 2009 Jun 23;180(13):E118-25.

(433) Stone R. Guam: deadly disease dying out. Science 1993 Jul 23;261(5120):424-426.

(434) Okle O, Stemmer K, Deschl U, Dietrich DR. L-BMAA induced ER stress and enhanced caspase 12 cleavage in human neuroblastoma SH-SY5Y cells at low nonexcitotoxic concentrations. Toxicol Sci 2013 Jan;131(1):217-224.

(435) Alonso V, Villaverde-Hueso A, Hens MJ, Morales-Piga A, Abaitua I, de la Paz MP. Increase in motor neuron disease mortality in Spain: temporal and geographical analysis (1990-2005). Amyotroph Lateral Scler 2011 May;12(3):192-198.

(436) Georgoulopoulou E, Vinceti M, Bonvicini F, Sola P, Goldoni CA, De Girolamo G, et al. Changing incidence and subtypes of ALS in Modena, Italy: A 10-years prospective study. Amyotroph Lateral Scler 2011 Nov;12(6):451-457.