



## Full length article

# Identification of risk factors associated with onset and progression of amyotrophic lateral sclerosis using systematic review and meta-analysis



Ming-Dong Wang<sup>a,b,\*</sup>, Julian Little<sup>a</sup>, James Gomes<sup>c,d,e</sup>, Neil R. Cashman<sup>f</sup>,  
Daniel Krewski<sup>a,c,g</sup>

<sup>a</sup> School of Epidemiology, Public Health and Preventive Medicine, Faculty of Medicine, University of Ottawa, ON K1H 8M5, Canada

<sup>b</sup> School of Life Science, Changchun Normal University, Changchun, Jilin 130032, China

<sup>c</sup> McLaughlin Center for Population Risk Assessment, University of Ottawa, Ottawa, ON, Canada

<sup>d</sup> Interdisciplinary School of Health Sciences, Faculty of Health Sciences, University of Ottawa, ON, Canada

<sup>e</sup> Environmental Health Research Unit, Interdisciplinary School of Health Sciences University of Ottawa, 25 University Pk., Ottawa, ON K1N 6N5, Canada

<sup>f</sup> Brain Research Centre, Department of Medicine (Neurology), University of British Columbia, Vancouver, British Columbia, Canada

<sup>g</sup> Risk Sciences International, Ottawa, ON, Canada

## ARTICLE INFO

## Article history:

Received 29 June 2016

Accepted 29 June 2016

Available online 1 July 2016

## Keywords:

Amyotrophic lateral sclerosis

Meta-analysis

Systematic review

Risk factor

Etiology

Onset

Progression

## ABSTRACT

Although amyotrophic lateral sclerosis (ALS) was identified as a neurological condition 150 years ago, risk factors related to the onset and progression of ALS remain largely unknown. Monogenic mutations in over 30 genes are associated with about 10% of ALS cases. The age at onset of ALS and disease types has been found to influence ALS progression. The present study was designed to identify additional putative risk factors associated with the onset and progression of ALS using systematic review and meta-analysis of observational studies. Risk factors that may be associated with ALS include: 1) genetic mutations, including the intermediate CAG repeat expansion in *ATXN2*; 2) previous exposure to heavy metals such as lead and mercury; 3) previous exposure to organic chemicals, such as pesticides and solvents; 4) history of electric shock; 5) history of physical trauma/injury (including head trauma/injury); 6) smoking (a weak risk factor for ALS in women); and 6) other risk factors, such as participating in professional sports, lower body mass index, lower educational attainment, or occupations requiring repetitive/strenuous work, military service, exposure to Beta-N-methylamino-L-alanine and viral infections. Risk factors that may be associated with ALS progression rate include: 1) nutritional status, including vitamin D deficiency; 2) comorbidities; 3) ethnicity and genetic factors; 4) lack of supportive care; and 4) smoking. The extent to which these associations may be causal is discussed, with further research recommended to strengthen the evidence on which determinations of causality may be based.

© 2016 Published by Elsevier B.V.

## 1. Introduction

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative condition affecting the voluntary motor nervous system (Sathasivam, 2010; Piaceri et al., 2011; Talman et al., 2009). The typical symptoms/signs in ALS patients include progressive atrophic weakness and spasticity of affected regions, and the spread of symptoms from the site(s) of onset. The smooth and striated

muscles of sphincters, the muscles responsible for ocular movement, and the sensory nerves (temperature, pain, taste, hearing, vision, olfaction) are usually spared. However, dementia, cognitive impairment and executive dysfunction have been detected in more than 50% of ALS patients (Phukan et al., 2007, 2011; Gordon et al., 2011b), especially the ALS patients with mutations in the *C9orf72* and *TDP43* genes (Cooper-Knock et al., 2012; Byrne et al., 2012; Ludolph et al., 2012; Seltman and Matthews, 2012). In most ALS cases, disease progression is rapid. The average survival time from diagnosis is less than 3 years, with affected individuals often dying of respiratory failure or inanition. Notably, a small proportion (~10%) of ALS cases survive for more than 10 years (Testa et al., 2004), and rare ALS cases have survived

\* Corresponding author at: School of Epidemiology, Public Health and Preventive Medicine, Faculty of Medicine, University of Ottawa, ON K1H 8M5, Canada.

E-mail addresses: myang015@uottawa.ca, mdwangforever@yahoo.ca (M.-D. Wang).

for more than 50 years (Tosi et al., 1994). Multiple ALS subsets and ALS variants have been described (Silani et al., 2011; Kim et al., 2009), indicating the heterogeneity of ALS phenotypes (Piaceri et al., 2011; Chio et al., 2011; Turner et al., 2013; Sabatelli et al., 2013). Different phenotypes have been reported among individuals with the same type of gene mutations, such as those of the *C9orf72* (Murray et al., 2011; Stewart et al., 2012), *SOD1* (Piaceri et al., 2011), *TDP43* and/or *FUS* genes (Mackenzie et al., 2011).

ALS is usually diagnosed within a year of the appearance of the first clinical signs (Mitchell et al., 2010). There is currently no specific diagnostic test for ALS. Instead, diagnosis relies upon consideration of differential diagnoses and the presence of unique clinical signs – upper and lower motor neuron findings confined to spinal and bulbar voluntary muscles. Pathological degeneration signs include lateral sclerosis of descending motor tracts in motor neuron cells in the brainstem and spinal anterior horns. Clinical diagnostic criteria for ALS, referred to as the El Escorial criteria, were proposed by the World Neurology Federation in 1994, and revised in 2000 and 2008 (Silani et al., 2011). According to these criteria, ALS cases are classified as definite, probable, or possible. The World Health Organization (WHO) categorized ALS as a major subset of motor neuron disease (MND), and all subsets of MND share an identical International Classification of Diseases (ICD) code. Therefore, in the biomedical literature, MND and ALS are often used interchangeably. Misdiagnosis or misclassification has been estimated to account for about 10% of total ALS cases (Silani et al., 2011).

ALS is a degenerative motor neuron disease that is terminal and irreversible. Disease severity can be measured using the ALS functional rating scales (ALSFRS), having a score ranging from 0–40, based on 10 basic functions in 4 key functional domains (swallowing, walking/self-care, communicating and breathing) (Anonymous, 1996). The disease course of ALS has recently been divided into 6 stages, progressing from stage 0 for no loss of function to stage 5 for death (Chio et al., 2013a,b). The progression to death of ALS is apparently slower for spinal ALS than for bulbar onset cases, with the age at onset being negatively associated with survival time (Chio et al., 2009a,b,c).

The crude incidence of ALS in Europe and North America, especially among whites, is quite stable, about 2–3 cases per 100,000 people per year (Cronin et al., 2007; Alonso et al., 2009). Although the incidence of ALS in Asia is generally lower, the highest incidences (50–100 fold greater than the global average) have been reported in Asia, specifically in Guam and the surrounding Pacific islands during the 1940–1960s (Plato et al., 2003; Okamoto et al., 2009b; Spencer et al., 2005; Waring et al., 2004; Steele, 2005; Yoshida et al., 1998). Populations of Hispanic or African origin display lower incidences of ALS (Cronin et al., 2007). Because of the short survival time of ALS patients, the mortality rate of ALS roughly reflects its incidence rate (Marin et al., 2011a).

Both the incidence and prevalence of ALS are greater in men than in women, the ratio being approximately 1.5:1 (McCombe and Henderson, 2010). Male sex is also associated with age of onset and ALS disease type (Gordon et al., 2011a). A preponderance of ALS in males has been reported for respiratory, flail arm, classic and pure lower motor neuron (p-LMN) phenotypes (Chio et al., 2011). This male preponderance has declined in recent decades, suggesting that changing exposure to environmental risk factors for ALS may explain part of this sex difference (Gordon et al., 2011a).

ALS is an age-associated neurodegenerative condition, having some similarities to chronic diseases such as Alzheimer's disease; however, unlike Alzheimer's disease, ALS is not strictly an aging-associated condition (Brody and Grant, 2001). The incidence of ALS increases with age, peaks at about 70 years of age, and then declines rapidly thereafter (Logroscino et al., 2008). Although ALS is a rare neurodegenerative condition, it has been reported that the

proportion of overall mortality due to ALS has increased in recent decades, more so in women than in men (Gordon et al., 2011a).

ALS cases have been classified as belonging to one of two categories – familial or sporadic, although this classification system is now being challenged. About 10% of sporadic ALS cases are caused by monogenic mutations (Chio et al., 2012). Unlike many other chronic diseases, such as various cancers, sporadic ALS cases are not randomly distributed in the general population. Geographical (Doi et al., 2010; Turabelidze et al., 2008; Malaspina et al., 2002; Ceroni et al., 1999; Poloni et al., 1997; Gunnarsson et al., 1996; Uccelli et al., 2007; Noonan et al., 2005), occupational (Gunnarsson et al., 1996; Barth et al., 2009; Rose and Brix, 2006), and conjugal (Poloni et al., 1997) ALS clusters have been observed, suggesting that shared environmental risk factors also play an important role in the etiology of ALS. Overall, genetic factors and environmental risk factors are considered to have equally important roles in the onset of ALS (Wingo et al., 2011; Al-Chalabi et al., 2010). ALS is one of most heterogeneous neurodegenerative conditions, in terms of etiology, phenotype and progression. Although many genetic and environmental risk factors have been associated with the onset of sporadic ALS in many observational studies, no single gene variant or environmental risk factor has been conclusively linked to the onset of sporadic ALS. To date, few reviews have assessed the risk factors associated with the progression of ALS. It is important for the management of ALS cases that appropriate diagnostic criteria be defined, and that known and unknown risk factors be characterized and described. The objectives of this study were to identify environmental and genetic risk factors that are associated with the onset or progression of ALS through systematic reviews and meta-analyses of observational studies.

## 2. Methods

### 2.1. Literature search

A literature search strategy was designed using a set of search terms for the disease itself, risk factors for the disease, and related terms (Supplementary material I). The search criteria were refined and optimized using PubMed (Hersi et al., 2016), and then used to search the scientific literature in other databases. The strategy focused on the searches of systematic reviews/meta-analyses and observational studies for risk factors associated with ALS disease occurrence and progression (Supplementary materials II–IV). Other databases searched included Medline, Embase, the HuGE Literature Finder (<https://phgkb.cdc.gov/HuGENavigator/startPagePubLit.do>), Toxiline, and Psynfo. All searches were initially conducted through to December 2013, with an update covering the period December 2013 to February 16, 2016.

### 2.2. Literature screening

Relevant articles were identified and exported into Distiller (DistillerSR, Evidence Partners, Ottawa, Canada) software for further evaluation and review. Additional articles from manual searches were added to this database when appropriate. The articles were screened based on title and abstract, using the inclusion criteria (disease terms, study types, human study, and article types) described in Supplementary materials I–IV. The full text of the included articles was further examined for relevance according to priori criteria focusing on disease terms, study types, population information, statistical methods, results, and conclusions (Supplementary materials I–IV). Relevant data from all articles retained following full-text screening were extracted in

Distiller using a pre-designed data collection algorithm. A random sample of 5% screened articles at all screening levels was cross-examined by second reviewer in order to validate the process of article screening and data extraction.

### 2.3. Literature assessment and meta-analyses

The identified systematic reviews were assessed using the AMSTAR criteria (Shea et al., 2007). If these reviews were of moderate or good quality, they were updated with new observational studies; otherwise the review was done *ab initio*. The observational studies were assessed according to previously developed criteria (Turner et al., 2011; Stroup et al., 2000). If more than 3 observational articles for a given risk factor were found, then a meta-analysis of those studies was conducted with RevMan5.1 software (<http://tech.cochrane.org/revman/download>). The odds ratio (OR) for ALS risk due to exposure to individual environmental risk factors was used as the summary measure of risk. The primary analysis was based on random effects (RE) modelling, with a fixed effects (FE) model run as a secondary analysis. Heterogeneity across included studies was estimated by  $\tau^2$ ,  $\chi^2$ , and  $I^2$  (Higgins and Thompson, 2002). Forest plots and relevant supporting statistics were also examined. Funnel plots (Begg's test) were used to evaluate possible publication bias including small study effects. Meta-analyses for subgroups, such as disease subtype, sex, and article quality, were considered if at least three articles were available in a category. ALS associated genetic factors were summarized from the existing ALS associated gene website (Lill et al., 2011), and from searches of PubMed and other databases. If the number of included observational studies is less than three, the information from these studies was described individually.

## 3. Results

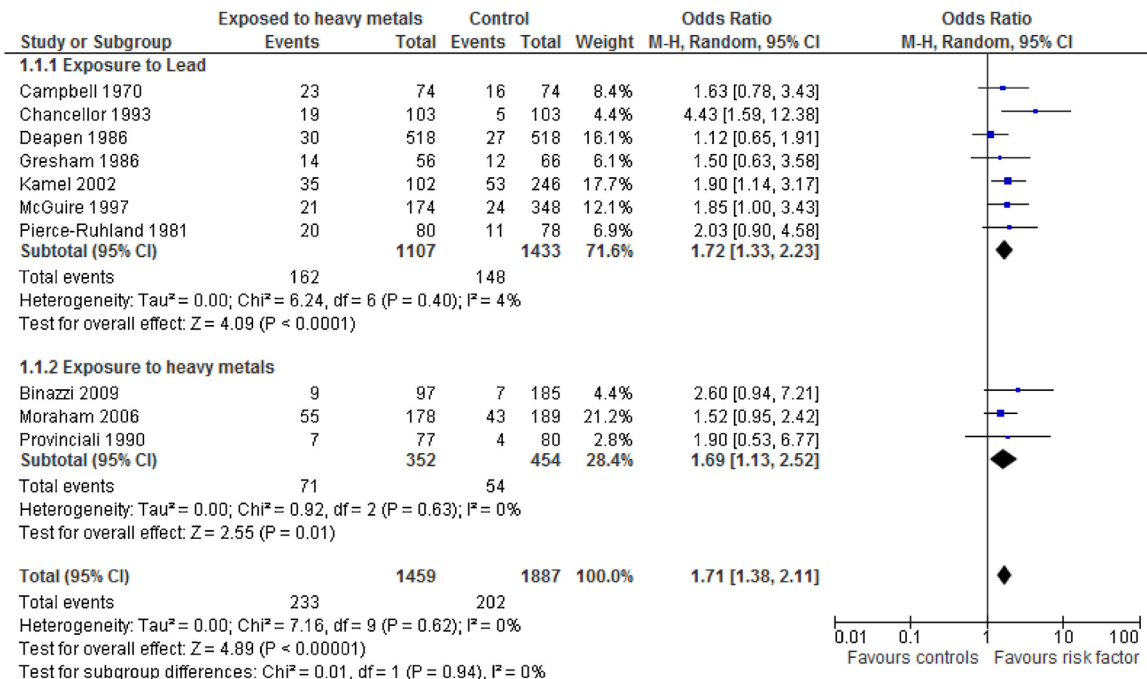
### 3.1. Risk factors associated with onset of ALS

#### 3.1.1. Risk factors identified in available systematic reviews (SR) and meta-analyses (MA)

Two SR/MA articles had moderate quality AMSTAR scores: one SR/MA assessed the association between exposure to chemicals and ALS (Sutedja et al., 2009a,b), the other between occupation and ALS (Sutedja et al., 2007). Neither SR identified any clear association between these risk factors and ALS. One comprehensive SR/MA suggested that smoking was not a strong risk factor for ALS (Alonso et al., 2010a,b), was re-analysed with all identified observational studies (see below). Two recent SR/MAs found that previous exposure to pesticides was significantly associated with an increased risk of developing ALS (Kamel et al., 2012; Malek et al., 2012), were also re-analysed with additional observational studies. A SR/MA of observational studies examined the association between exposure to electromagnetic fields and ALS (Zhou et al., 2012). As this association may have been confounded by other related risk factors such as by occupation and electronic shock, data synthesis and discussion in depth have been provided in present study. Seven new SR/MA articles were identified in the update (Supplementary material V) and are discussed below. There appear to be unrealistic expectations that SR/MA can establish or disprove a causal relationship between risk factors and diseases (Wang and Little, 2016), and most SR/MAs may have not included sufficient information to draw firm conclusions (Belbasis et al., 2016).

#### 3.1.2. Risk factors identified in primary observational studies

In the first round search, a total of 88 observational studies related to environmental risk factors was identified. In the updated



**Fig. 1.** Previous exposure to lead/heavy metals is associated with increased risk of developing ALS. Exposure data were extracted from ten case-control studies, and the association between ALS and previous exposure to lead against controls was synthesized by meta-analysis using random effects model. No evidence of heterogeneity across included studies was observed, nor was there evidence of significant publication bias (data not shown).

search, 45 additional observational studies were identified (Supplementary material V). This updated information is included in the results and discussion section of the present paper.

**3.1.2.1. Exposure to heavy metals.** Individuals with an occupational history of exposure to heavy metals, particularly lead, were at an increased risk for ALS (Supplementary material VI). Based on seven case-control studies, meta-analysis showed that the odds ratio of having a history of exposure to lead was significantly increased for ALS patients when compared to controls [RE model, OR = 1.72 (1.33, 2.23)] (Fig. 1). There was little evidence of heterogeneity across studies ( $I^2 = 20\%$ ), or of small study effects (funnel plot, not shown).

The second most common heavy metal putatively associated with ALS is mercury. There have been case reports of ALS or ALS-like symptoms associated with exposure to mercury (Callaghan et al., 2011; Johnson and Atchison, 2009; Praline et al., 2007; Zhou et al., 2014), although the epidemiological investigation for this association has not been extensively conducted (Gresham et al., 1986).

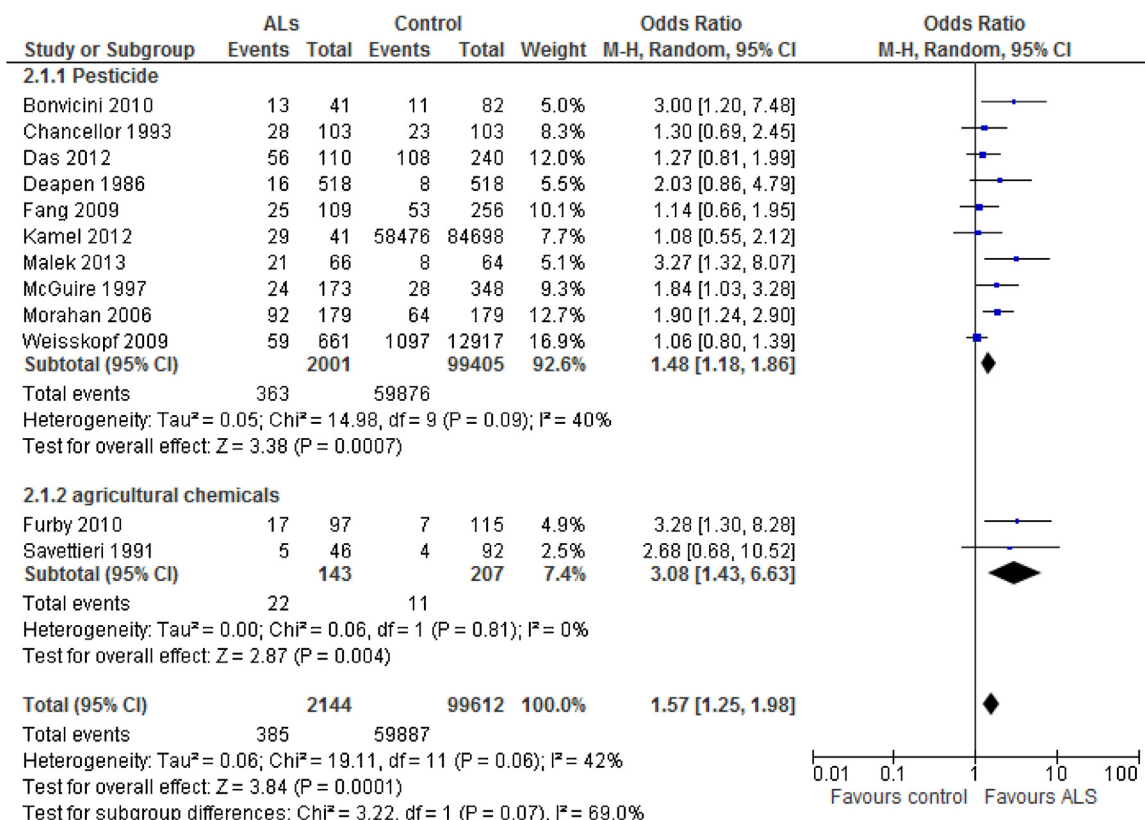
Previous exposure to other heavy metals, such as selenium, magnesium, aluminum, manganese and cadmium, may be also associated with an increased risk of developing ALS (Ahmed and Wicklund, 2011; Vinceti et al., 2012; Cannon and Timothy Greenamyre, 2011).

**3.1.2.2. Agricultural chemicals.** Previous exposure to agricultural chemicals, especially to pesticides, appears to be associated with ALS (Supplementary material VII). Meta-analysis showed that the occupation of farming was associated with ALS [RE, OR = 2.18 (1.23–3.84)], but with significant heterogeneity across the 10 observational studies included ( $I^2 = 76\%$ , data not shown).

However, living in a rural area or having an occupation in the agricultural sector may not be associated with onset of ALS. This association might be confounded by exposure to agricultural chemicals. A meta-analysis of 12 case control studies revealed that the OR for ALS in individuals with a history of exposure to pesticides, including agricultural chemicals, was increased by about 50% and showed moderate heterogeneity in these studies [RE model, OR = 1.57 (1.25–1.98),  $I^2 = 42\%$  (Fig. 2)], consistent with three meta-analyses (Kamel et al., 2012; Malek et al., 2012; Kang et al., 2014).

The blood levels of two pesticides were significantly increased in ALS compared to non-ALS individuals (pentachlorobenzene: OR = 2.21; 95% CI, 1.06–4.60;  $p = 0.04$ ; and cis-chlordane: OR = 5.74; 95% CI, 1.80–18.20;  $p = 0.005$ ) (Su et al., 2016).

**3.1.2.3. Organic solvents.** Previous exposure to organic solvents has been reported to be a risk factor for ALS (Table 1) (McGuire et al., 1997; Chancellor et al., 1993; Fang et al., 2009; Morahan and Pamphlett, 2006; Park et al., 2005). However, these studies did not specify what solvents were involved. Thus, articles that used the term 'solvent' were selected for meta-analysis. The OR of having ALS in individuals exposed to solvents, was found to be increased by more than 40%, with no significant heterogeneity across these 7 case-control studies [RE model, OR = 1.43 (1.10–1.86),  $I^2 = 16\%$  (Fig. 3)] or publication bias and small study effects (data not shown). Studies identified in the update specified the nature of the solvents involved. In a study of 101 cases and 110 controls in which measurements of persistent environmental pollutants in whole blood or plasma were made, positive association between ALS and detectable polychlorinated biphenyls 175 (OR = 1.81; 95% CI, 1.20–2.72;  $p = 0.005$ ) and detectable polychlorinated biphenyls 202



**Fig. 2.** Previous exposure to pesticides/agricultural chemicals is associated with ALS. The relationship between previous exposure to pesticides (10 articles) or agricultural chemicals (two articles) and ALS was estimated using RevMan 5.1 with random effects model. There was no evidence of significant publication bias (data not shown).

**Table 1**

Summary of studies related to exposed to solvents and ALS onset.

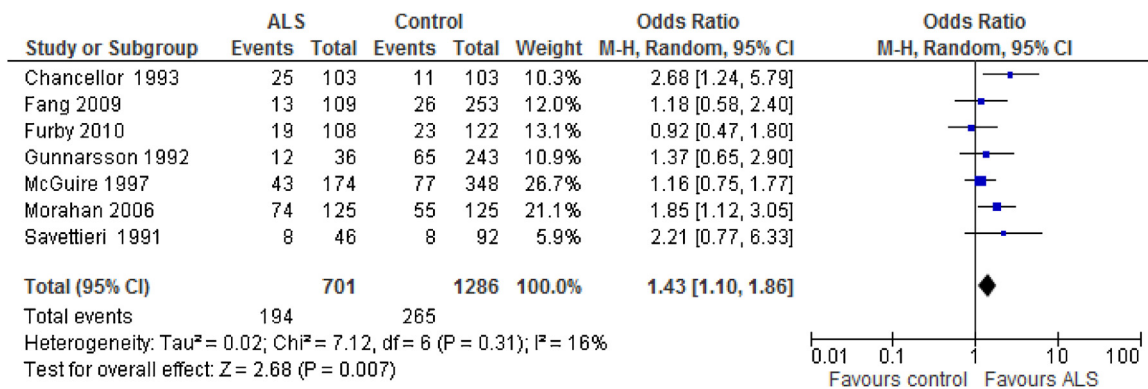
Author, Year, country	Study type	Participants	Characteristics	Solvent
McGuire, 1997, USA	Case control	1.4-year period 1990–1994 2. ALS cases aged 18 or elder years who were newly diagnosed with ALS in western Washington State were identified through a surveillance system, but the cases who lacked a telephone 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 ( $\pm 5$ 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. 4. 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.	Solvents: Since there was no agreement for a significant association between self-reported and panel assessed, the results not extracted. But cleaning solvent/degreasers was associated with ALS with agreement: Panel assessment(unexposed/exposed): cases = 131/43, controls = 271/77, OR = 1.9(1.1–3.3)
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 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).	Solvents: 1.case/control 5 case only 20 control only 6 neither 72 OR = 3.3(1.3–10). 2. cases (exposed/unexposed) = 25/78, controls = 11/92
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.	Solvents (e.g., toluene or xylene) 1. cases(exposed/unexposed) = 13/96 2. control = 26/237; 3. cases = 109, control = 253.
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 $\pm$ 13.1 (SD) 52.5 $\pm$ 14.9 (SD) Weight (Kg) – F: 66 $\pm$ 19 (SD) 65 $\pm$ 10 (SD) Weight (Kg) – M: 82 $\pm$ 13 (SD) 86 $\pm$ 17 (SD)	Solvents (case-control): 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.
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	Solvents, Exposed/non-exposed: cases = 8/38, controls = 8/84, OR = 2.14(0.6–7.2)
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	Solvent: 1. OR = 1.76(1.19–2.59) Logistic analysis. 2. Univariate analysis: Solvent/chemical (exposed/non-exposed): cases, 92/86; control, 64/115, OR = 1.92(1.26–2.93); men, cases, 74/51; controls, 55/70, OR = 1.85(1.12–3.04); Women, cases, 18/35 controls, 9/45; 2.57(1.05–6.31)
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 were 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% 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	Solvent: It seems that exposed to solvent is associated with PMA, not ALS. 1. Relations (men and women together) between exposure to solvents and trauma (fracture or surgery) less than 10 years before onset of PMA: No trauma (exposed/unexposed): cases = 11/12, controls = 65/178, OR = 2.5(1.0–6.5). Trauma(exposed/unexposed): cases = 6/7, controls = 50/79, OR = 1.8(0.5–5.4) 2. Relations (men and women together) between exposure to solvents and trauma

**Table 1** (Continued)

Author, Year, country	Study type	Participants	Characteristics	Solvent
		4. The subjects were asked about their eating habits during the 1960s, 1970s, and 1980s.	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.	(fracture or surgery) less than 10 years before onset of ALS or PBP. No Trauma (exposed/unexposed): cases = 12/24, controls = 65/178, OR = 1.4(0.6–3.0) Trauma(exposed/unexposed): cases = 5/15, CONTROLS = 50/79, OR = 0.7(0.2–2.1). Organic agents or Organic molecules yes/no (reference): Cases = 19/89, controls = 23/99, OR = 0.957 [0.476–1.927], p = 0.903.
Furby, J 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 orthopaedic 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.	1. Age at interview: Patients, M = 68 SD = 18.0, range[34–86]; Controls, M = 65; SD = 18.0 range[31–84] 2. Sex men/women: Patients, 59/49; Controls, 68/54.	
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. ICD-9 (1989–1998) code 335.2 or ICD-10 (1999–2004) code G12.2 (motor neuron disease) was used. 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.	1. The 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.	Solvents(including chemicals and acids, not include organic solvents, a typical misclassification, cohort) Chemicals/acids/solvents No, cases (1014), P.Y = 12,070, Ref, adj.RR1 Ref, adj.RR2 Yes, cases(142), P.Y = 1508, RR = 1.05(0.86–1.29), RR2 = 1.04 (0.73–1.49)
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).	1. Motor neuron disease: white men, 3851; white women, 2152; non-white men, 203; non-white women, 141; total, 6,347.2. Total deaths: white men, 1,479,921; white women, 803,110; non-white men, 203,862; Non-white women, 127,453; total, 2,614,346.	Solvents – organic agents(cohort). 1. Solvents: Total deaths 972,505; MND death, 1994, RR = 1.16(1.01–1.34) 2. Benzene: Total deaths, 624,524; 1356, RR = 1.14(0.97–1.33)
Buckley J, 1983, England	Other	Not mention	Cases = 356 + 346 + 335, from mortality data	Exposed to solvent, chemicals, via occupation (cross-section) Leather workers, Clothing workers Construction workers Furnace forge, foundry, rolling mill workers, Textile workers, Painters and decorators, Food, drink and tobacco workers, Professional technical workers, artists, Transport and communications workers, Engineering and allied trades workers, NEC, Sales workers, Paper and printing workers, Mines and quarrymen

(OR = 2.11; 95% CI, 1.36–3.27; p = 0.001) were observed (Su et al., 2016). In a study of 51 cases and 51 controls in which residential histories were geocoded and linked with databases on air pollution measurements, residential exposure to aromatic solvents was positively associated with ALS (OR = 4.27, 95% CI: 1.09–16.79); (Malek et al., 2015).

**3.1.2.4. Exposure to formaldehyde.** In an analysis of a cohort comprising about one million participants, an increased relative risk of ALS mortality among adults who reported that they had ever been occupationally exposed to formaldehyde [RR = 1.34 (95% CI: 0.93–1.92) for full cohort; RR = 2.47 (95% CI: 1.58–3.86) after excluding individuals with missing duration of formaldehyde exposure] has been reported (Weisskopf et al., 2009). A strong



**Fig. 3.** 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. There was no evidence of significant publication bias (data not shown).

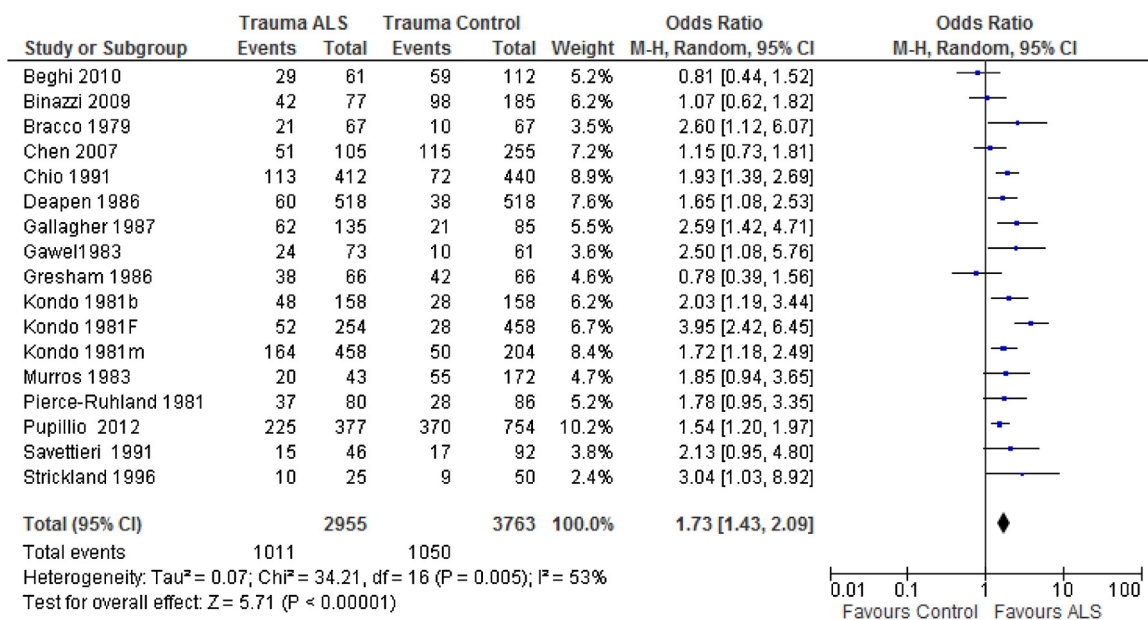
dose-response relationship between years of formaldehyde exposure and ALS mortality was identified (trend  $p = 0.0004$ ). The RRs for those reporting  $<4$  years, 4–10 years, and  $>10$  years of exposure formaldehyde to the unexposed were 1.5, 2.1 and 4.1, respectively (Weisskopf et al., 2009). This same research team reported that in a cohort of almost 800,000 men, those who had had jobs with a high probability of exposure to formaldehyde had almost 3-fold greater ALS mortality than in those with no such exposure (Roberts et al., 2015). However, in a cohort study of over 11,000 garment workers exposed to formaldehyde with over 414,000 person-year follow-up, in which there were only eight deaths due to ALS. The association of formaldehyde exposure with ALS mortality was not significant. The major limitation of this study is the sample size (Pinkerton et al., 2013).

**3.1.2.5. Previous physical trauma/injury including head trauma/injury.** Meta-analysis of 15 case-control studies (Supplementary material VIII) showed a positive association between ALS and

previous trauma, with moderate heterogeneity [RE model,  $OR = 1.73$  (1.43–2.09,  $I^2 = 53\%$ )] (Fig. 4). Old trauma, defined as trauma that happened at least 5 years prior to ALS diagnosis, was also found to be associated with ALS based on the 4 studies included [RE model,  $OR = 1.40$  (1.06–1.86),  $I^2 = 33\%$ ]. Further subgroup analyses revealed that both previous head trauma [RE model,  $OR = 1.27$  (1.02–1.57),  $I^2 = 9\%$ ] and bone fractures (data not shown) were associated with increased risk of ALS.

These conclusions were further supported by recent results showing a strong association between ALS and physical trauma in a large population based case-control study (Seals et al., 2016) or cerebrovascular injury in a new cohort study (Turner et al., 2015).

**3.1.2.6. Lower education.** The pooled OR for ALS from three studies (Supplementary material VIII) in individuals with lower education compared to higher education, was 2.04 (95%CI: 1.58–2.62), with no apparent heterogeneity among these three studies (RE model,  $I^2 = 0\%$ ).



**Fig. 4.** Previous trauma is associated with increased ALS risk. Articles related to trauma including any injury causing medical attention and ALS were searched from multiple literature sources. Data related to injury among ALS and controls from 17 studies in 16 articles was extracted, the synthesized OR was computed based on the odds of injury among ALS cases and controls based on meta-analysis using the random effects model in RevMan 5.1. Similar OR estimates were also obtained with the fixed effects model (data not shown).

**Table 2**

Summary of studies related to the association of ALS onset with other comorbidities.

Author, Year, Country	Study type	Participants	Characteristics	Pre-morbidity
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. 3. 334 patients and 538 controls were included. 4. In the blood sample study, 303 patients and 2100 controls (from other sources) were included.	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 0.5–1.0, $p = 0.02$ ); 2. Moreover, patients had a lower BMI (ALS BMI = 25 ± 3.5, control = 26 ± 3.6, OR = 0.9, 95% CI 0.9–1.0, $p = 0.001$ ). 3. TC and LDL were significantly lower, and HDL was significantly higher in ALS patients. 4. The LDL/HDL ratio was significantly lower in patients with ALS (in women, OR = 0.4, 95% CI 0.3–0.6, $p < 0.001$ ; in men, OR = 0.5, 95% CI 0.4–0.6, $p < 0.001$ ).
Beghi E, 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, controls were not exactly matched. 2. Age, marital, education, no difference between P and C.	BMI BMI. cases: 25.3, SD 4.5; controls: 26.1, SD 4.1 Details were not provided in this paper
Okamoto K, 2009, Japan	Case control	1. Study period: 2000 and 2004 2. Recruited 153 ALS patients aged 18–81 years with disease duration of 3 years.	1. Sex ratio = 60.3/39.7 in both groups 2. Average age: Cases = 63.7 ± 9.2; control = 63.4 ± 10.6	BMI: cases were slimmer than controls: cases = 22.2 ± 0.3; controls = 23.3 ± 0.3; $P = 0.04$

Table 2 (Continued)

Author, Year, Country	Study type	Participants	Characteristics	Pre-morbidity
		3. 306 gender- and age-matched controls were randomly selected from the general population during the study period. 4. Controls: 418 out of 505 completed the entire questionnaires. For cases, should be 100%		
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	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)
Freeman, 2005, USA	Cohort Study	Follow up at least one year	1.9 million population whites only	Comorbidity-cancer 1. There is no significant increase for the risk of ALS among all cancer patients. SMR = 1.0 2. The risk of ALS in melanoma patients is significantly higher than expected SMR = 1.6(1.1–2.6), 32 ALS cases were found in melanoma survivors. The excess incidence of ALS was also observed in tongue tumor survivors SMR = 2.7(1.1–5.7), but only 7 ALS cases were observed.
Turner MT, J 2012, UK	Cohort Study	1. Experimental group: with CVD. Oxford Record Linkage Study (ORLS),10 comprising admissions in the former Oxford National Health Service (NHS) region from 1963 to 1998 (ORLS1) and from 1999 to 2008 (ORLS2). 2. Control, no CVD. A cohort without a record of CHD was built consisting of all those admitted to hospital with a wide range of non-cardiovascular conditions comprising mainly minor medical and surgical conditions. This cohort was used as a 'reference cohort', presumed to approximate the general population in its risk of major diseases.	1. 1351 ALS cases in No CHD population from England dataset 2. 1313 ALS cases in CHD population from England dataset 1. 282 ALS cases in no CVD population from ORLS1 and ORLS2 2. 93 ALS cases in CHD population from ORLS1 and ORLS2	Comorbidity-cardiovascular disease 1. RR for ALS = 1.14(1.05–1.22) in non-cardiovascular diseases versus vascular diseases, similar results obtained from additional two smaller study populations 2. RR for PD or MS is not different between two groups.
Huisman MHB, 2011, Netherlands	Case control	1. All newly diagnosed patients and all patients diagnosed before January 2006 and still alive on January 1, 2006, were selected. 2. Controls should be of the same sex and age, plus or minus 5 years. 3. Patients and controls were	1. January 1, 2006, and May 31, 2000. 2. 41 patients (6.4%) had at least one family member with ALS and were, therefore, classified as having FALS. 3. The remainder (594 patients) were classified as having SALS. Relatives of patients have an elevated risk of ALS compared to	Comorbidity-Cardiovascular-infarction 1. Dementia, PD, or DPD was not significantly increased in SALS or FALS patients' relatives. 2. However, the incidence of myocardial infarction in SALS or FALS' relatives is significantly decreased, compared to that in the relatives of controls.

**Table 2** (Continued)

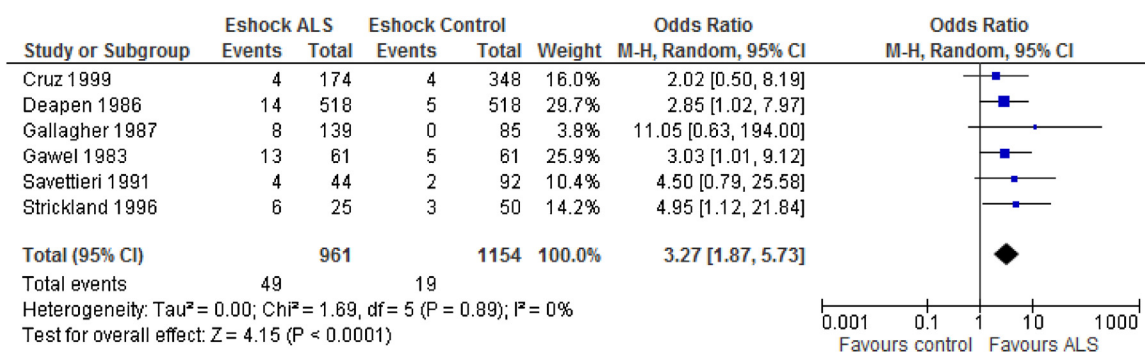
Author, Year, Country	Study type	Participants	Characteristics	Pre-morbidity																																			
		asked to fill in structured questionnaires. 4. No difference between controls and patients with regards to the sex ratio, age.	controls (any relative 2.42; 95% CI 1.65–3.57). 4. Definite + probable cases: 461 (74%); possible cases: 159 (25), 7 cases are missing. 5. Bulbar onset: 198 (31%) 6. Males: 388(61%) in cases, 935(58%) in controls 5. Onset ages: 60.5 ± 11.4; diagnostic age: 61.8 ± 11.4	<table><tr><th></th><th></th><th>N</th><th>Relatives</th><th>Affected</th><th>Ratio</th><th>OR 95%Ci</th></tr><tr><td>MI</td><td>SALS</td><td>594</td><td>7679</td><td>553</td><td>0.072</td><td>0.96(0.79-0.94)</td></tr><tr><td></td><td>CONTROLS</td><td>1566</td><td>20925</td><td>1749</td><td>0.084</td><td></td></tr><tr><td>CONTROLS</td><td>FALS</td><td>41</td><td>606</td><td>31</td><td>0.051</td><td>0.61(0.43-0.86)</td></tr><tr><td></td><td>CONTROLS</td><td>1,616</td><td>21584</td><td>1805</td><td>0.084</td><td></td></tr></table>			N	Relatives	Affected	Ratio	OR 95%Ci	MI	SALS	594	7679	553	0.072	0.96(0.79-0.94)		CONTROLS	1566	20925	1749	0.084		CONTROLS	FALS	41	606	31	0.051	0.61(0.43-0.86)		CONTROLS	1,616	21584	1805	0.084	
		N	Relatives	Affected	Ratio	OR 95%Ci																																	
MI	SALS	594	7679	553	0.072	0.96(0.79-0.94)																																	
	CONTROLS	1566	20925	1749	0.084																																		
CONTROLS	FALS	41	606	31	0.051	0.61(0.43-0.86)																																	
	CONTROLS	1,616	21584	1805	0.084																																		
Mitchell JD, 1995, UK	Case control	1. 128 cases were seen in a hospital from a target population of 1.8 million from 1988–1993. 2. 2 controls for each case matched by sex, age with 2 years, and residence. One control with neuron disease, one control was a healthy individual 3. The questionnaires were filled by a nurse	1. Men/women ratio = 1.51 2. Mean age at onset was 61 for men, 64 for women years old respectively.	Comorbidity-thyroid disease Have ever had thyroid disease: RR = 3.02(1.05–8.66)																																			
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 8 controls: 52 men, 26 women. 8.1 Mean age at interview for both groups were the same – 52 years. 6. No race data	Genetic-Neurological disorders: Neurological disorders were associated with ALS. 32 cases, but only 17 cases with neurological disorders in relatives.																																			
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. 3. 334 patients and 538 controls were included.	1. Median age, range: for cases, 60 (24–82); for controls, 59(29–89). 9 female, N(%): For case, 145(43), for controls, 246(46). 9.1 Bulbar onset: 86 (27%) 4. The response rate of the participants in the questionnaire study was 80%	LDL/HDL ratio 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 0.5–1.0, p = 0.02); 2. Moreover, patients had a lower BMI (OR = 0.9, 95% CI 0.9–1.0, p = 0.001). 3. TC and LDL were significantly lower, and HDL was significantly higher in ALS patients. 4. The LDL/HDL ratio was significantly lower in patients with ALS (in women, OR = 0.4, 95% CI 0.3–0.6, p < 0.001; in men, OR = 0.5, 95% CI 0.4–0.6, p < 0.001).																																			

Table 2 (Continued)

Author, Year, Country	Study type	Participants	Characteristics	Pre-morbidity
Mattsson P, 2012, Sweden	Case control	4. In the blood sample study, 303 patients and 2100 controls (from other sources) were included. Linked data: Population Registry, Military Service Conscription Register, Swedish Cause of Death register. Men were born in 1951–1965, and lived in Sweden in the years of 17th birth day.	1. Population size: 809 789 total population (122 ALS cases), 686815 has been tested the fitness (85 ALS cases) 2. Nested control were selected	physical fitness(which is determined by genetic factors) ALS is statistically associated with body weight adjusted physical fitness( $p = 0.01$ , OR = 1.98, 1.32–2.97), but not physical fitness per se ( $p = 0.09$ , OR = 1.01), not body weight( $p = 0.06$ , OR = 0.98), height( $p = 0.18$ , OR = 0.98), muscle strength (handgrip, $P = 0.19$ , OR = 1.0), resting heart rate( $p = 0.09$ , OR = 1.02). Body weight adjusted knee extension is also associated with increased ALS ( $p = 0.05$ , OR = 1.15(1.00–1.33))
Sørensen, 2010, Finland	Case control	1. Performed this population-based case-control study in the northern part of Denmark, which has approximately 1.8 million inhabitants. 2. selected 10 control subjects for each case matched on sex, birth year, and calendar time, from source population	Cases Controls Age in years, mean (SD)* 66.2 (12.0) 66.2 (12.0) Male sex, n (%) 302 (54.3) 3020 (54.3)	Cases Controls Use of statins, n (%) Never 477 (85.6) 4754 (85.5) 1 (reference) Ever 79 (14.2) 806 (14.5) 0.96 (0.73, 1.28) Duration of statin use <3 y 50 (9.0) 455 (8.1) 1.08 (0.78, 1.51) >=3 y 29 (5.2) 351 (6.3) 0.79 (0.52, 1.20)
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. 3. 334 patients and 538 controls were included. 4. In the blood sample study, 303 patients and 2100 controls (from other sources) were included.	1. Median age, range: for cases, 60 (24–82); for controls, 59(29–89). 10 Female, N(%): For case, 145(43), for controls, 246(46). 10.1 Bulbar onset: 86(27%) 4. The response rate of the participants in the questionnaire study was 80%.	Statin-Cholesterol drug 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 0.5–1.0, $p = 0.02$ ); 2. Moreover, patients had a lower BMI (OR = 0.9, 95% CI 0.9–1.0, $p = 0.001$ ). 3. TC and LDL were significantly lower, and HDL was significantly higher in ALS patients. 4. The LDL/HDL ratio was significantly lower in patients with ALS (in women, OR = 0.4, 95% CI 0.3–0.6, $p < 0.001$ ; in men, OR = 0.5, 95% CI 0.4–0.6, $p < 0.001$ ).

**3.1.2.7. Strenuous occupation.** A meta-analysis of seven case-control studies (Supplementary material VIII) (Fang et al., 2009; Beghi et al., 2010; Binazzi et al., 2009; Gallagher and Sanders, 1987; Kihira et al., 2007; Provinciali and Giovagnoli, 1990; Strickland et al., 1996) showed a statistically significant association between strenuous work and ALS (RE model OR = 2.70, 95% CI: 1.97–3.69), with no significant heterogeneity across the studies.

**3.1.2.8. Participating in professional sports, but not recreational sports is associated with ALS.** Six case-control studies were included in the meta-analysis of the association between physical activity or participating in sports and ALS [Supplementary material VIII (Okamoto et al., 2009b; Beghi et al., 2010; Longstreth et al., 1998; Vanacore et al., 2010; Veldink et al., 2005b; Pupillo et al., 2012)]. The results showed that participating in recreational sports or



**Fig. 5.** Previous electric shock is associated with ALS. The association between alcohol drinking (10 articles) and ALS was estimated using the random effects model in RevMan 5.1, providing evidence of a significant increase in risk overall. There was no evidence of significant publication bias (data not shown).

other physical activities was not associated with an increased risk of ALS [RE model, OR = 0.90, 95%CI: 0.78–1.03;  $I^2 = 24\%$ ]. In contrast, ever participating in an organized athletic club in high school or college, or in professional sports, was likely to be associated with ALS (Beghi et al., 2010; Longstreth et al., 1998; Veldink et al., 2005b; Pupillo et al., 2012) [RE model, OR = 1.35, 95% CI: 1.11–1.65.  $I^2 = 3\%$ ].

**3.1.2.9. Lower body mass index (BMI).** Cohort studies revealed that BMI at baseline was inversely associated with ALS risk (O'Reilly et al., 2013). For each 5-unit increase in BMI within the overweight and obese body weight range, the ALS risk was 21% lower (95% CI: 14–27%). This association persisted among never smokers. Excluding the first seven years of follow-up, the associations remained unchanged, suggesting that weight loss from undiagnosed ALS does not fully explain the findings (O'Reilly et al., 2013). Our meta-analyses from case-control studies supported this inverse association [Table 2, RE model, OR =  $-0.24(-0.34-0.14)$ ,  $I^2 = 0\%$ ].

**3.1.2.10. Previous electric shock.** The meta-analysis of all 6 case-control studies related to electric shock (Supplementary material IX) showed that previous experience of electric shock was strongly associated with increased risk of ALS [RE model, OR = 3.27 (1.87–5.73),  $I^2 = 0\%$ ] (Fig. 5).

**3.1.2.11. Cigarette smoking.** The results of a meta-analysis of 20 case-control studies (Supplementary material X) did not support a statistically significant association between smoking and increased risk of ALS [RE: OR = 0.97 (0.88–1.08), FE: OR = 0.94 (0.87–1.01)] for ever smokers versus never smokers. However, a mild excess relative risk (RR) of developing ALS in female ever smokers compared to non-smokers was observed, based on three cohort studies, with no significant heterogeneity [RE: RR = 1.34 (1.17–1.55);  $I^2 = 12\%$ ]. Other subgroup meta-analyses, such as former versus current smokers among male ALS cases, did not uncover any significant associations between smoking and ALS (data not shown).

However, most studies from Europe and the United States showed that smoking was a risk factor for ALS (Okamoto et al., 2009b; Wang et al., 2008, 2011a,b; Gallo et al., 2009; Kamel et al., 1999; Fang et al., 2006; Berg et al., 2011; Alonso et al., 2010a,b; Schmidt et al., 2010; Sutedja et al., 2007; Weisskopf et al., 2004). A statistically significant association between ever smoking (compared with never smoking) and the risk of ALS was identified in females (RR = 1.7; 95% CI: 1.3–2.1), but not in males (RR = 0.9; 95% CI: 0.7–1.0) using meta-analysis (Alonso et al., 2010a,b). It was estimated that the overall risk of ALS in current smokers (compared with never smokers) was about 1.3 times that of non-smokers (RR = 1.28; 95%CI: 1.0–1.7). This association between

smoking and ALS supports the hypothesis that smoking might be a weak risk factor for ALS (Fang and Ye, 2010; Armon, 2009). However, the inverse (or null) association between lung cancer (Gibson et al., 2016; Catala-Lopez et al., 2014; Freedman et al., 2014), which is strongly associated with smoking, and ALS seems to further support a conclusion of weak association between ALS and smoking, even if there is an association.

**3.1.2.12. Military service.** As the term 'military service' was excluded when observational studies were screened to identify modifiable risk factors associated with ALS for the general public, we summarize the main observations of the association between ALS and military service here. Excess ALS cases among Gulf veterans, especially aged less than 45 years, were observed in two independent reports, involving comparison with undeployed military personnel (Horner et al., 2003) or the general population (Haley, 2003). The excess cases occurred within the first decade after the Gulf war (Horner et al., 2008), and might be associated with special deployment locations (Miranda et al., 2008). This excess risk of ALS was diminished when using the 13-year follow-up data for the Gulf veterans (Barth et al., 2009).

The risk of ALS due to military service was significantly higher than in controls [RR = 1.53 (1.12–2.09)] (Weisskopf et al., 2005a,b), this observation is supported by following study (Bryan et al., 2016). Although military service itself was not a strong risk factor for ALS [RR = 1.34 (0.87–2.06)] when compared to controls, 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)]. The risk of ALS for war participants increased with the number of wars (RR = 1.57, 1.74, or 1.97) for one, two, and three or more wars, respectively]. Individuals who served during World War II may be at increased risk of ALS (HR: 1.47; 95% CI: 1.13, 1.91) (Weisskopf et al., 2015). The risk of ALS found in a Danish cohort was highest in the decade immediately following the end of military service (Seals et al., 2016).

These observations indicated that the military service associated risk factors, such as chemicals used in battle fields, might be responsible for the increased risk of ALS (Beard and Kamel, 2015).

**3.1.2.13. Viral infection.** A higher prevalence of spinal fluid enterovirus genome has been detected in ALS patients in some studies (Vandenberghe et al., 2010; Cermelli et al., 2003; Berger et al., 2000; Jubelt, 1992), but not in others (Jubelt and Lipton, 2004; Nix et al., 2004). An association between polio virus infection and ALS has also been proposed (Esik et al., 2004), but has never been confirmed.

**3.1.2.14. Cardiovascular factors.** Genetic variants of VEGF, the gene encoding a growth factor for vascular endothelial cells, have been

**Table 3**

Summary of studies related to alcohol intake and ALS onset.

Author, Year, Country	Study type	Participants	Characteristics	Others
Nelson LM, 2000, USA	Case control	1. Control: randomly selected. The combined response rate for eligible controls identified and contacted by using both methods was 79 percent (348/441). 2. Patients had to be residents of one of the three counties, aged 18 years or older, and newly diagnosed with ALS during the 4- year study period.	N/A	Alcohol coffee 1. Alcohol: no association had been identified. cases(yes/no) = 73/88, controls = 129/192. 2.No relation was observed between caffeine intake and the risk of ALS (relative to the lowest quartile of caffeine intake, the odds ratios adjusted for age, gender, respondent type, education, and smoking were 0.9, 1.0, and 0.9 for the second through fourth quartiles of caffeine intake, respectively). Alcohol drinking, stress, type A behaviour, less intake of green vegetables 1. Current drinking: 69.1% for cases, 68.0% for controls OR = 1.1 (0.7–1.5). Cases (yes/no) = 106/47, control = 208/98. 2. Self-assessed stress: 61.9% for cases, 47.4% for controls OR = 2.9 (1.3–2.7) 3. Type A: 44.2% for cases 19.6% for controls OR = 2.9 (1.9–4.5) 4. Interaction between green/yellow vegetables: 50.8% for cases 70.6% for controls OR = 2.5 (1.7–3.7) 5. Not confounded between type A and vegetable consumptions.
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 2000–2005 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	Alcohol drinking (repeat) Alcohol drinking is not associated with ALS.
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	Alcohol drinking, 1. Drinking: cases = 35.3%, controls = 31.4%. cases(yes/no) = 108/198, controls = 131/287.
Okamoto K, Japan, 2009	Case control	1. Study period: 2000 and 2004 2. Recruited 153 ALS patients aged 18–81 years with disease duration of 3 years. 3. 306 gender- and age-matched controls were randomly selected from the general population during the study period. 4. Controls: 418 out of 505 completed the entire questionnaires. For cases, should be 100%	1. Sex ratio = 60.3/39.7 in both groups 2. Average age: Cases = 63.7 ± 9.2; control = 63.4 ± 10.6	
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, controls were not exactly matched. 2. Age, marital, education, no difference between P and C.	Alcohol 1. Work intensity (Yes/no, P < 0.05): Strenuous/all, P = 8/53, C = 4/108; 2. Sport intensity (yes/no, P > 0.05): strenuous/all others, p = 8/53, C = 19/93 3. Professional sport(yes/no, p = 0.04): P = 3/58, C = 0/112 4. Alcohol(yes/no): P, 18/43; C, 24/88 Details were not provided in this paper
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	Alcohol Study A: Alcohol drinks: RR = 0.90, 59.3% in male cases, 67.1% in male controls. RR = 1.18, 13.7 in female cases, 10.9 in female controls. Cases(yes/total) = 307/712, control(yes/total) = 335/712 Study B: Alcohol: RR = 0.78 for males, RR = 1.04 for females. Cases(yes/no) = 60/98, controls = 71/87.
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	Alcohol(exposed/non-exposed): cases = 18/28, controls = 37/55, OR = 0.76(0.4–1.4)
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.	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.	alcohol, Formaldehyde, exposed to organic agents Overall smokers non-smokers 1. Exposed to Adhesive: 36 23 1.5 (0.8–2.7) 22 15 1.2 (0.5–2.6) 14 8 2.4 (0.9–6.9) 2. Dyes or printing inks: 37 21 1.4 (0.8–2.7) 22 13 1.1 (0.5–2.4) 15 8 2.5 (0.9–7.2) 3. Cutting/cooling/lubricating oils: 43 32 1.8 (1.0–3.3) 32 22 1.2 (0.6–2.5) 11 10 6.3

**Table 3** (Continued)

Author, Year, Country	Study type	Participants	Characteristics	Others
		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.		(1.9–20.4) 4. Antifreeze or coolants: 23 17 1.6 (0.8–3.3) 17 10 0.9 (0.3–2.4) 6 7 6.3 (1.6–24.1) 5. Degreasers or cleaning agents: 59 28 1.0 (0.6–1.7) 40 20 0.8 (0.4–1.5) 19 8 1.5 (0.5–4.2) 6. Mineral spirits/white spirits: 20 13 1.7 (0.8–3.7) 13 7 1.0 (0.4–2.8) 7 6 4.1 (1.2–14.4) 7. exposed to formaldehyde: Never, controls 204, cases 89; Ever, controls 49, cases 20, OR = 0.8(0.5–1.5) Alcohol: no association: cases (yes/no) = 29/71, controls = 42/214.
kamel F, 1999, USA	Case control	1. Cases were recruited from two referral center between 1993–1996. 2. Cases were eligible to participated if they had received their first diagnosis within two years. 3. Speak English and able to participate the study, i.e., no mental and physical disability. 4. 223 sequential cases, 154 cases were eligible, and final 109 cases finished the studies. 5. Population Controls were identified through telephone screening. 354 eligible controls were contacted, 270 agreed to participated, and 256 finish the questionnaires	1. Conducted between 1993–1996 2. 109 cases, 256 controls, 3. 95% whites 4. 61% men, 39 women in both cases and controls 5. No average age, but case and control were age matched, the ages range from 35 to 80 in both groups.	
de jong, Holand, 2012	Case control	1. 494 incident ALS patients diagnosed with ALS on or after January 1, 2006. 2. 255 prevalent ALS patients diagnosed before January 1, 2006, but alive after that date. 3. An overlap of 178 patients between the prevalent and previously studied patient groups. 4. A referral ALS population (n = 366 or 359) diagnosed between January 1, 2001, and December 31, 2005.	1. There was no difference between incident and prevalent ALS patients in terms of sex ratio, age at onset, disease duration and education level. 2. Bulbar type in incident (34.8%) versus prevalent (24.8%). 3. Elementary education level in control (5.7%) versus in all ALS patients (10.8%).	Incident case(494) prevalent cases(255) control (1599) never 15.8 20.2 9.7 Former drinkers 5.3 7.4 5.1 Current 78.9 72.4 85.2

associated with ALS (Terry et al., 2004; Barbeito et al., 2010; Lambrechts et al., 2009; Wang et al., 2007). Beneficial vascular factors, such as lower LDL/HDL, have been associated with an increased risk of ALS (Sutedja et al., 2011; Turner et al., 2012; Kim et al., 2011; Paganoni et al., 2011, 2012; Dorst et al., 2011).

**3.1.2.15. Beta-N-methylamino-L-alanine (BMAA).** ALS clusters in the Gulf desert and in New Hampshire have been attributed to the intake of BMAA (Cox et al., 2009; Caller et al., 2009, 2012). Over the last few years, several ecological studies showed an association between the spatial distribution of BMAA and the incidence of ALS in France (Masseret et al., 2013) and the USA (Field et al., 2013).

**3.1.2.16. Alcohol.** A meta-analysis of existing observational studies showed that alcohol drinking was not associated with increased ALS risk (data not shown, Table 3). However, a recent study indicated an inverse association (incident patient group: OR = 0.52, 95% CI: 0.40, 0.75) (de Jong et al., 2012), suggesting alcohol may be a protective factor against ALS.

**3.1.2.17. Coffee.** Based on a case-control study of 377 cases and three control groups, drinking coffee may be a protective factor against ALS (Beghi et al., 2011). The ORs for ALS comparing to neurologic, non-neurologic, and controls recruited through family practices 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 (Beghi et al., 2011). However, this observation was not seen in a pooled analysis of data on 1279 cases

**Table 4**

Monogenic mutations related to ALS onset.

Gene	Protein name and function	transmission	Gene location	Mean onset age (y)
ALS2	Amyotrophic lateral sclerosis 2	AD	2q33.2	1
C9orf72	Unknown	AD	9p21.2	
DCTN1	Dynactin 1 (p150, glued homolog, Drosophila)	AD	2p13	55
FUS	Fusion (involved in t(12;16) in malignant liposarcoma)	AD	16p11.2	45
OPNT	Optineurin	AD	10p13	51
SETX	Senataxin, ALS 4	AD	9q34.13	18
SOD1	Superoxide dismutase 1, soluble (amyotrophic lateral sclerosis 1 (adult))	AD/AR	21q22.11	47
SPG11	Spastic paraplegia 11	AR	15q14	16
TAF15	TAF15 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 68 kDa		17q11.1-q11.2	50
TARDBP	TAR DNA binding protein	AD	1p36.22	55
UBQLN2	Ubiquilin	X-linked	Xp11.21	41
VAPB	VAMP (vesicle-associated membrane protein)-associated protein B and C	AD	20q13.33	44

occurring in over one million people included in 5 cohort studies (Fondell et al., 2015).

**3.1.2.18. Vitamin E, vitamin C and unsaturated fatty acids.** Intake of supplemental vitamin E, but not other vitamins, has been associated with reduced risk of developing ALS, which is consistent with ALS being related to reactive oxygen species (Veldink et al., 2007; Wang et al., 2011a,b). A case-control study showed that intake of vitamin E was associated with a reduced ALS risk (OR=0.4, 95% CI: 0.2–0.7,  $p=0.001$ ) (Veldink et al., 2007). 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 (Wang et al., 2011a,b). The most recent cohort study showed that the age-adjusted relative risk for ALS was significantly lower in subjects with higher serum Vitamin E levels (RR=0.56, 95%CI: 0.32–0.99,  $p=0.046$ ) when compared to those with lower levels (Michal Freedman et al., 2013). In addition, intake of vitamin C, carotenoids, and unsaturated fatty acids may also reduce ALS risk, or delay ALS onset (Fitzgerald et al., 2013, 2014).

**3.1.2.19. Fruit and vegetables.** Two case-control studies from Japan reported an inverse association between intake of fruit/vegetables and the risk of ALS (Okamoto et al., 2009a,b).

**3.1.2.20. Chicken soup.** One cohort study showed that the risk of ALS, among people who consumed chicken soup versus never having consumed chicken soup, was significantly lower after adjusting for age and smoking [RR=0.94 (0.66–1.34) for one serving, 0.79 (0.55–1.13) for 2–3 servings, and 0.58 (0.36–0.94) for more than 4 servings per day], with a statistically significant dose-dependent reduction trend (trend  $p=0.0006$ ) (Morozova et al., 2008).

### 3.1.3. Genetic factors

Many genetic risk factors have been associated with ALS. Several internet sites have been established to collect information from genetic studies of ALS (He et al., 2015). The most comprehensive web site is the ALSOD site (Lill et al., 2011; Abel

et al., 2012). It not only collects complete gene mutation information and basic demographic information of patients, but also provides researchers with some simple data analytic results and related lists of literature references. Most of the genetic information presented below was summarized from this website. However, we also ran an article search for some risk factors (the search strategy for genetic factors is outlined in Supplementary material III).

**3.1.3.1. Genetic factors with a Mendelian inheritance pattern.** Although ALS is a relatively rare neurodegenerative condition, 658 mutations in 126 genes have been linked to ALS. ALS mutant genes could be found in every chromosome, except Y chromosome. Of these 126 genes, 32 mutated genes (Table 4) may be transmitted in a Mendelian inheritance pattern based on 1094 ALS patients with complete genetic data (<http://alsod.iop.kcl.ac.uk/Statistics/report.aspx>, access 2016), explaining about 10% of diagnosed ALS cases (Chio et al., 2012). This list of gene mutations is growing (Abel et al., 2012). The two most common monogenic mutations are of SOD1 and C9orf72 among white ALS patients, accounting for 25% and 35% of familial cases, and 2% and 6% of sporadic cases, respectively (Chio et al., 2012; Wijesekera and Leigh, 2009; Pfister et al., 2013; Robberecht, 2000; van Blitterswijk et al., 2012b). C9orf72 mutations have been reported in populations of European origin, but very rarely in Chinese or Japanese populations (Turner et al., 2013; van Blitterswijk et al., 2012b; Chen et al., 2016). The C9orf72 mutation is thought to have arisen in Scandinavia several thousand years ago (Smith et al., 2012; Ratti et al., 2012). The manner by which environmental factors might interact with this gene has not been well investigated (Figuerola-Romero et al., 2012), but has suggested that the expression of these genes might be modulated by environmental risk factors via DNA methylation (such as on FUS gene) (Kaneb et al., 2012). Unlike most inherited diseases, the onset of inherited ALS usually occurs late in life, at around 50 years of age [calculated from ALSOD website (ALSOD, Jan. 2014)], and can occur as late as 85 years of age (Aggarwal and Nicholson, 2005). The inheritance penetration is much lower than 100% for most genes of this type. Therefore, the onset time of inherited ALS might be subject to modification by

**Table 5**  
Meta-analyses for the associations between gene variants and ALS onset.

Gene	Chromosome site	Name Function	Variants	Studies included	Pooled OR	Conclusion
ANG	14q11.1	Angiogenin	Rs11701 (G/T)	3 p, 2 n, 5 no association	1.13(0.93–1.40) $i^2=75$	Heterogeneity is high across studies, conclusion might be not appropriate
APEX1	14q11.2	APEX nuclease (multifunctional DNA repair enzyme) 1	Rs1130409 (T/G)	1 n, 3 no association	0.78(0.62–0.97) $i^2=20$	Gene variant may reduce the risk for ALS
APOE	19q13.2	Apolipoprotein E	Rs429358 (C/T)	1 n, 8 no association	0.94(0.79–1.12) $i^2=44$	May be associated with age of onset, presentation and survival
ATXN2	4q32.1	Glutamate receptor	Rs10025251 (C/T)	6 no association	0.99(0.91–1.08) $i^2=0$	Strongly associated with it
GRIA2	4q32.1	Glutamate receptor	Rs10025251 (C/T)	6 no association	0.99(0.91–1.08) $i^2=0$	Seems no association with this variant
GWA_9p21	9p21.2	unknown	Rs2814707 (A/G)	6 p, 1 n, 9 no association	1.24(1.18–1.30) $i^2=70$	Associated with ALS, but heterogeneity is high across studies.
HFE	6p21.3	Haemochromatosis oxidative stress	Rs1799945 (G/C)	4 p, 2 no association	1.72(1.20–2.48) $i^2=74$	Also associated with dementia
PON1	7q21.3	Paraoxonase 1 metabolism of xenobiotics	Rs662 (G/A)	1 p, 7 no association	1.10(1.00–1.22) $i^2=32$	Contrary to prediction, this variant is associated with ALS, although heterogeneity across studies
PON2	7q21.3	Paraoxonase 1 metabolism of xenobiotics	Rs7493 (C/G?)	1 p, 5 no association	1.10(0.86–1.42) $i^2=34$	This variant is associated with ALS, but inconsistent with a recent meta-analysis. I favour this association here.
PON3	7q21.3	Paraoxonase 1 metabolism of xenobiotics	Rs10487132 (G/A)	1 p, 4 no association	1.09(0.97–1.23) $i^2=44$	May not be strongly associated with variant
UNC13A	19p13.11	protein unc-13 homolog A, Neurotransmitter release	Rs12608932 (C/A)	8 p, 9 no association	1.15(1.10–1.21) $i^2=31$	Also a survival modifier. Diekstra FP, Neurobiol Aging. 2012 Mar; 33(3): 630.e3-8
VEGFA	6p12	Vascular endothelial growth factor	Rs699947	1 p, 9 no association	1.06(0.98–1.16) $i^2=0$	May not be strongly associated with ALS

**Table 6**

Studies related to PolyQ expansion in ataxin-2 gene.

Author, Year, Country	ALS or MND Diagnosis	Sporadic, Familial sex, Case, Control and PolyQ information	Main results and Conclusion
Included studies			
Conforti, 2012, Italy	ALS, El 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 <i>ATXN1</i> alleles in sALS cohort harboured a $\geq 32$ 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 <i>ATXN2</i> , 22 X $\geq 28Q$ 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).
Corrado, 2011, Italy	ALS, El Escorial. Screened SOD1, TDP-43, FUS, regionally matched controls	1. 232 ALS (219 sALS, 13 fALS): 1 $\times$ 24Q; 1 $\times$ 27Q, 0 $\times$ 28Q; 1 $\times$ 29Q; 0 $\times$ 30Q; 3 $\times$ 31Q; 1 $\times$ 32Q; 2 $\times$ 33Q; 1 $\times$ 37Q. 2. 395 controls: 3 $\times$ 24Q; 6 $\times$ 27Q, 1 $\times$ 28Q; 4 $\times$ 29Q; 1 $\times$ 30Q; 0 $\times$ 31Q; 0 $\times$ 32Q; 0 $\times$ 33Q; 0 $\times$ 37Q.	1. Significantly association. 2. No observation for symptoms of ataxia, dementia or other atypical features.
Daoud H, 2011, France/Qubec, Canada	1. ALS, El 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: $\geq 32$ , 9; 2. 95 fALS: $\geq 32$ , 2; 3. 471 unrelated controls. Range from 19–32. 19–37 in patients. 24 of 471 healthy controls (5.1%) harboured 1 intermediate <i>ATXN2</i> 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 ( $\geq 27$ ), no difference( $p=0.09$ ). The $p$ becomes significant when compared within the range of $\geq 29$ . 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 $\geq 32$ remove, the difference is still significant (14 from cases, 3 from controls, OR = 4.03(1.15–14.11)) for the range between 29 and 31. 2. Length is not associated with age at onset ( $\geq 29$ ) in cases were no $\geq 29$ cases.
Elden, 2010, USA	ALS, recruited in PA and NJ, no recruitment time, no case control design	1. 915 ALS. 6 $\times$ 24Q; 1 $\times$ 25Q; 0 $\times$ 26Q; 22 $\times$ 27Q; 1 $\times$ 28Q; 2 $\times$ 29Q; 4 $\times$ 30Q; 7 $\times$ 31Q; 8 $\times$ 32Q; 2 $\times$ 33Q. 2. 980 Controls: 4 $\times$ 24Q; 4 $\times$ 25Q; 2 $\times$ 26Q; 11 $\times$ 27Q; 0 $\times$ 28Q; 1 $\times$ 29Q; 0 $\times$ 30Q; 2 $\times$ 31Q; 0 $\times$ 32Q; 0 $\times$ 33Q	1. CAG intermediate repeat expansion is associated with ALS. 2. The repeat might be a modulator.
Gellera, 2012, Italy	ALS, El Escorial, SOD1 free, for all, fALS also screened ANG, TDP-43, FUS, C9ORF72 (carriers of fALS cases-separated)	1. 658 SALS: 4 $\times$ 24–26Q; 10 $\times$ 27Q, 4 $\times$ 29Q; 3 $\times$ 30Q; 7 $\times$ 31Q; 6 $\times$ 32Q; 2 $\times$ 33Q. No $>33Q$ . 2. 41 fALS-G(carriers): 1 $\times$ 27–29Q; 1 $\times$ 30Q. No others 3. 102 fALS-Unknown: 2 $\times$ 24–26Q; 1 $\times$ 27–29Q; 1 $\times$ 30Q. 4.231 Sporadic ataxic patients: 5 $\times$ 24–26Q; 6 $\times$ 27–29Q; 2 $\times$ 30Q. No others. 4. 551 Health controls: 6 $\times$ 24–26Q; 18 $\times$ 27–29Q; 1 $\times$ 30Q; 1 $\times$ 31Q. No others.	1. The frequency of <i>ATXN2</i> alleles with 27–30 repeats was similar in SALS and control subjects. 2. Fifteen SALS subjects carried $\geq 31$ CAG repeats. This difference was statistically significant ( $p=0.0014$ ). No alleles with $\geq 34$ CAG were found. 3. In fALS, the distribution of <i>ATXN2</i> alleles was similar to control subjects.
Gispert, 2012, Germany	ALS, European ALS clinics (diagnostic criteria),	1. 559 ALS (included 89 fALS, 1 $\times$ 32Q): 1 $\times$ 24Q, 1 $\times$ 25Q; 0 $\times$ 26Q; 15 $\times$ 27Q; 0 $\times$ 28Q; 1 $\times$ 29Q; 3 $\times$ 30Q; 0 $\times$ 31Q; 3 $\times$ 32Q; 0 $\times$ 33Q; 0 $\times$ 34Q; 1 $\times$ 35Q. 2. 1378 controls: 9 $\times$ 24Q, 1 $\times$ 25Q; 0 $\times$ 26Q; 48 $\times$ 27Q; 1 $\times$ 28Q; 5 $\times$ 29Q; 1 $\times$ 30Q; 1 $\times$ 31Q; 0 $\times$ 32Q; 0 $\times$ 33Q; 1 $\times$ 34Q; 0 $\times$ 35Q. 3. 1142 PD: 6 $\times$ 24Q, 2 $\times$ 25Q; 1 $\times$ 26Q; 37 $\times$ 27Q; 1 $\times$ 28Q; 5 $\times$ 29Q; 0 $\times$ 30Q; 2 $\times$ 31Q; 1 $\times$ 32Q; 0 $\times$ 33Q; 0 $\times$ 34Q; 3 $\times$ 37Q; 1 $\times$ 39Q; 1 $\times$ 40Q.	1. In 559 sporadic ALS patients from Central Europe, the association of <i>ATXN2</i> expansions ( $30 \leq \text{polyQ} \leq 35$ ) with ALS was highly significant. 2. The study of 1490 patients with Parkinson's disease (PD) showed an enrichment of <i>ATXN2</i> 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 ( <i>ATXN3</i> ) and ALS risk.
Lahut, 2012, Turkey	ALS, no other information	1. 236 ALS. 4 $\times$ 24Q; 1 $\times$ 25Q; 3 $\times$ 27Q; 1 $\times$ 28Q; 1 $\times$ 29Q; 1 $\times$ 31Q; 3 $\times$ 32Q. 2. 420 control. 3 $\times$ 24Q; 0 $\times$ 25Q; 1 $\times$ 27Q; 0 $\times$ 28Q; 0 $\times$ 29Q; 0 $\times$ 31Q; 0 $\times$ 32Q.	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.
Lee T, 2011, Multiple European countries	ALS, El 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.

1. El Escorial. Recruited from 2008–2010

Table 6 (Continued)

Author, Year, Country	ALS or MND Diagnosis	Sporadic, Familial sex, Case, Control and PolyQ information	Main results and Conclusion
Ross, USA, Canada, 2011		1. 532 als: $\geq 27Q$ , 33(6.2%); $\geq 31Q$ , 8(1.5%). 2. 4877 control: $\geq 27Q$ , 197(4.0%); $\geq 31Q$ , 9(0.2%). 3. 642 FTD: $\geq 27Q$ , 31(4.8%); $\geq 31Q$ , 9(0.5%). 4. 1530 CE: $\geq 27Q$ , 56(3.7%); $\geq 31Q$ , 3(0.2%). 5. 514 PSP: $\geq 27Q$ , 24(4.7%); $\geq 31Q$ , 4(0.8%). 6.702 PD: $\geq 27Q$ , 28(4.0%); $\geq 31Q$ , 2(0.3%).	1. ALS, for $\geq 27$ , OR = 1.58(1.08–2.31); for $\geq 31$ , OR = 5.57(1.95–15.88). 2. FTD, for $\geq 27$ , OR = 1.20(0.82–1.76); for $\geq 31Q$ , OR = 1.94(0.51–7.37) 3. for AD, for $\geq 27$ , OR = 0.96(0.70–1.33); for $\geq 31Q$ , OR = 2.17(0.40–11.96) 4. For PSP, for $\geq 27$ , OR = 1.20(0.78–1.85); for $\geq 31Q$ , OR = 5.83(1.74–19.52) 5. For PD, for $\geq 27$ , OR = 0.95(0.63–1.43); for $\geq 31Q$ , 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?
Soraru, 2011, Italy	ALS, El Escorial, recruited from 01 of 2004–08 of 2010. Did not mention control match	1. 247 ALS. $\geq 24Q$ , 17(6.8%). $3 \times 24Q$ , $1 \times 26Q$ , $6 \times 27Q$ , $2 \times 30Q$ , $1 \times 31Q$ , $4 \times 32Q$ 2. 256 control: $\geq 24Q$ , 6(2.3%). $1 \times 24Q$ , $2 \times 27Q$ , $1 \times 28Q$ , $2 \times 31Q$ .	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.
Van Damme, 2011, Belgium/Netherlands	1. ALS, El Escorial. 1995–2010, neurological conditions free normal controls	1. 1845 SALS. $\geq 32$ , 10(5 $\times$ 32, 2 $\times$ 33, 1 $\times$ 34, 1 $\times$ 36, 1 $\times$ 39; 0.5%); (31,4; 30, 5; 29,9; 28, 1; Scientific guess from fig. we can further guess 27 repeat) 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.)	1. $p = 0.0006$ for repeat $\geq 32$ between ALS and control. No difference for $\leq 31$ (22–31, or 27–31 or 29–31). 2. ROC curve show a cutoff $\geq 29$ yield greatest sn, spn.28 out of 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, El Escorial, 05-2004-06-2010. Excluded fALS, Community controls matched by age, sex, race from same period.	1. 345 sALS (254 spinal, 91 bulbar). $\geq 24$ , 15; $< 24$ , 330; $\geq 27$ , 12; $< 27$ , 333; $\geq 28$ , 11; $\geq 29$ , 8; $\geq 31$ , 4. 2. 350 controls (17–30). $\geq 24$ , 8; $< 24$ , 342; $\geq 27$ , 4; $< 27$ , 346; $\geq 28$ , 3; $\geq 29$ , 2; $\geq 31$ , 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 $\geq 27$ . 3. Mean age of onset (for $\geq 31$ ), is longer than ( $< 31$ ): $44.5 \pm 8.5y$ versus $51.57 \pm 12.51$ , $p = 0.197$
Liu, 2013, China	ALS, El Escorial, whole China, did not state the recruitment time, no control match information	1. 1067 als: $4 \times 26Q$ ; $4 \times 27Q$ ; $3 \times 28Q$ ; $6 \times 29Q$ ; $3 \times 30Q$ ; $17 X > 30Q$ ( $6 \times 31Q$ ; $5 \times 32Q$ ; $3 \times 33Q$ ; $2 \times 34Q$ ; $1 \times 35Q$ ). 2. 506 healthy: $1 \times 26Q$ ; $2 \times 27Q$ ; $4 \times 28Q$ ; $0 \times 29Q$ ; $7 \times 30Q$ ; $0X > 30Q$ . $3 > 6$ fALS, no.	1. Association of ALS with ataxin-2 intermediate CAG repeats was confirmed. 2. No clinical manifestation associated with repeat observed.
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.
Multiple use of same sample			
Bonini, 2011, USA	Same as Elden	Title Model organisms reveal insight into human neurodegenerative disease: ataxin-2 intermediate-length polyglutamine expansions are a risk factor for ALS	Main results 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
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
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.

environmental factors. Several rarer gene mutations causing the onset of ALS in children have also been reported (ALSOD, Jan. 2014)

**3.1.3.2. Genetic polymorphisms.** Identifying the associations with polymorphic genetic variants in individuals with ALS has been an important research topic for decades. In addition to the ALSOD website, the ALSgene website mainly focuses on the information of gene polymorphisms identified in ALS cases. This website provides a list (total number is 22 as of May 2016) of top polymorphisms with strong association ( $P < 0.0001$ ) with ALS concluded by the results of at least one meta-analysis (Table 5). Of these 22 polymorphisms, 14 of them may increase ALS risk with mean OR between 1.12 and 1.56, whereas remaining 8 tested polymorphisms may decrease ALS risk with mean OR between 0.66 and 0.88. Establishing replicated associations between ALS and a gene polymorphism has proved difficult. For example, the association between the polymorphisms in gene PON1 reported in a few early studies has not been confirmed by a large scale meta-analysis based on pooling samples from multiple groups, including the samples from those early studies (Wills et al., 2009); this same conclusion was reached in subsequent studies (Ricci et al., 2011; van Blitterswijk et al., 2012a; Chen et al., 2012; Lee et al., 2015). Similar inconsistent results regarding the association between polymorphisms in VEGF (vascular endothelial growth factor) (Lambrechts et al., 2009), or H63D polymorphism in gene HFE (van Rheenen et al., 2013) and ALS have been reported. A literature search for peer-reviewed publications involving meta-analyses of the association between gene polymorphisms and ALS was conducted, and 26 articles with meta-analyses were identified (Chen et al., 2016; van Blitterswijk et al., 2012a; Lee et al., 2015; van Rheenen et al., 2013; Pan et al., 2016; Maiti et al., 2015; Pan et al., 2015; Lill et al., 2015; Zhao et al., 2016; Yang et al., 2015a,b; Chen et al., 2015; Smith et al., 2015; Keller et al., 2014; Ferrari et al., 2014; Diekstra et al., 2014; Govone et al., 2014; van der Zee et al., 2014; Wang et al., 2014a,b,c; Li et al., 2014; Fogh et al., 2014; Goris et al., 2014; ALSGEN Consortium et al., 2013; Ingram et al., 2012), but the question is that these conclusions drawn in these studies are reproducible in larger studies.

**3.1.3.3. ATXN2 gene.** The results of a meta-analysis of observational studies showed that the odds of having intermediate degree CAG repeat expansion in the ATXN2 gene among ALS patients were about 4 times higher than those of the controls (Table 6 and Fig. 6) (Wang et al., 2014a). Comprehensive reviews and meta-analyses about this topic recently conducted by other investigators have led to similar conclusions (Neuenschwander et al., 2014; Laffita-Mesa et al., 2013).

**3.1.3.4. Apolipoprotein E (APOE).** The APOE  $\epsilon 4$  allele may not be a risk factor for ALS (Govone et al., 2014; Praline et al., 2011; Zetterberg et al., 2008), unlike for Alzheimer's disease (Maiti et al., 2015). An association was reported between the  $\epsilon 4$  allele and the occurrence of bulbar-onset ALS among men, but not women (Praline et al., 2011).

**3.1.3.5. Early onset of balding (Androgenetic alopecia) and ALS.** Alopecia is a highly heritable condition. A cohort study of 51,529 US men found that the individuals with extensive alopecia had about 3-fold increased risk (out of 42 diagnosed ALS cases, 18 reported moderate, 11 reported extensive alopecia) for ALS compared with those with no alopecia, and the risk of ALS increases linearly with severity of alopecia ( $p$  trend = 0.02) (Fondell et al., 2013). This observation may help interpret the male preponderance of ALS.

## 3.2. Risk factors associated with progression of ALS

One systematic review of risk factors related to the progression of ALS was identified (Chio et al., 2009a,b,c). The risk factors from this review have been summarized and updated based on publications that have since become available, focusing on modifiable risk factors. Details of the search strategy and summary can be found in Supplementary materials II and III. We also identified one new meta-analysis and four new observational studies for ALS progression in the update search (Supplementary material V).

### 3.2.1. Age at onset

Age at the onset of ALS was inversely related with survival time (Chio et al., 2009a,b,c). For every increase of 10 years in the age at onset, the HR (hazard ratio) for survival time was reduced by about 40% (Alonso et al., 2010a,b; Kamel et al., 2008; Pastula et al., 2009). For some early onset cases, the disease progression rate was slower, or even imperceptible for up to 40–50 years (Tosi et al., 1994). It is believed that these cases might be caused by environmental factors, some of which are discussed in this paper. Some environmental factors are modifiable and others are not, and the presence or absence of these factors affects disease progression (Doi et al., 2006).

### 3.2.2. Sex

Most studies have not observed sex effects on ALS progression (Chio et al., 2009a,b,c). However, higher mortality and shorter survival time in women with ALS was reported (Chio et al., 2009a,b,c; Kamel et al., 2008).

### 3.2.3. Ethnicity

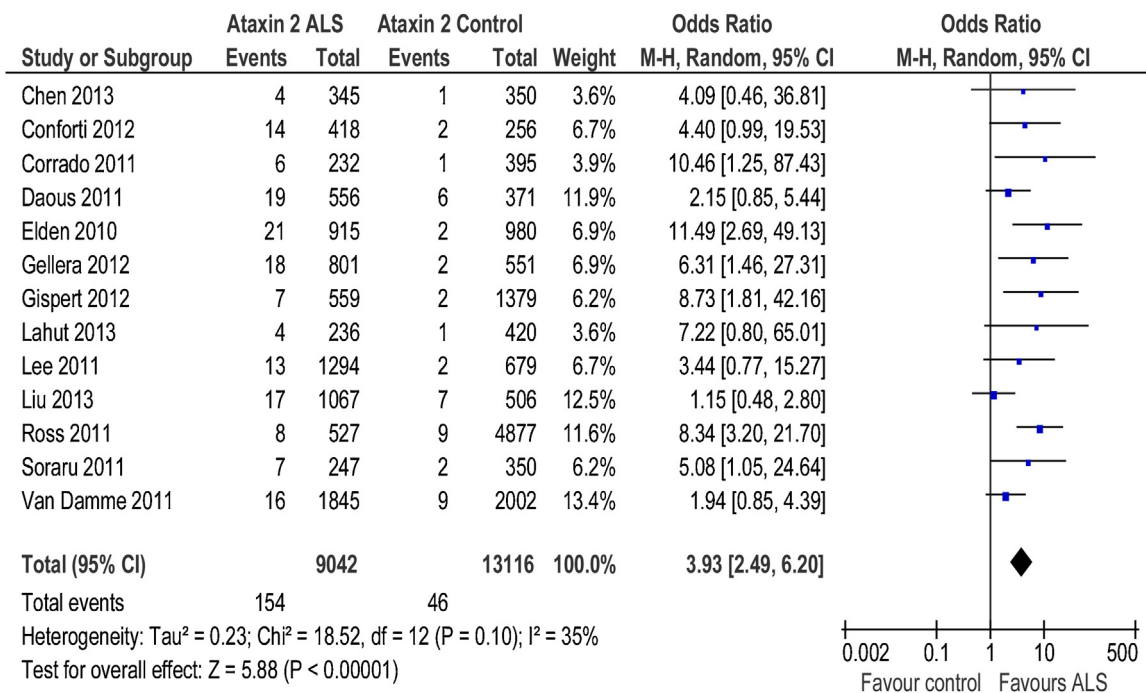
Ethnic Indians in Malaysia demonstrated a shorter interval between onset of symptoms and diagnosis, and shorter median survival time, when compared to non-Indians (Goh et al., 2011). However, analysis of Indians with ALS in Northern India revealed that among 1153 classic ALS patients, the overall median survival duration was  $114.8 \pm 25.9$  (SE) months, with a range of 3–194 months, longer than their White counterparts (Nalini et al., 2008). Patients of North African origin were significantly younger, and had a shorter duration of disease, when compared to other ethnic groups (European, North African, Oriental, Balkan, Arab), and adjusted for age (Drory and Artmonov, 2007).

### 3.2.4. Onset site or ALS type

ALS bulbar onset is associated with a poorer prognosis than spinal onset. Compared to spinal onset, survival time among patients with bulbar onset has been estimated to be lower by about 40% (Pastula et al., 2009). In addition, the majority (about 60%) of young-adult patients showed pure upper motor neuron ( $p$ -UMN) signs confined to upper limbs, whereas among adult onset ALS cases, this proportion was less than 20%. Young-adult ALS with  $p$ -UMN phenotype had longer survival (74 months, 95% CI: 60.61–87.38) than did the classic phenotype (56 months, 95% CI: 48.65–63.34). In young-adult patients, a much higher proportion of men were in the  $p$ -UMN ALS group (5.8:1), whereas the ratio of men to women was 1.1:1 in the classic phenotype group ( $p = 0.01$ ) (Sabatelli et al., 2008). Among ALS phenotypes [classic, bulbar, flail arm, flail leg, pyramidal, respiratory, pure lower motor neuron ( $p$ -LMN) and  $p$ -UMN], the best outcomes were observed in  $p$ -UMN, pyramidal,  $p$ -LMN and flail arm phenotypes, and the worst in respiratory and bulbar phenotypes (Chio et al., 2011).

### 3.2.5. Progression rate

Disease progression rate is usually inversely associated with survival time. One good progression indicator is the interval



**Fig. 6.** 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 the ratio of intermediate CAG repeat among ALS and control subjects was synthesized with meta-analysis using random effects model. Similar results were also obtained when using the fixed effects model (data not shown).

between the first sign of onset and diagnosis. Studies have shown that every year of increased time to diagnosis was associated with a nearly one year longer survival time ( $HR = 0.77$ ) (Pastula et al., 2009).

### 3.2.6. Body mass index (BMI)

BMI reduction rate from the BMI before disease onset to the time of the first visit is also a good indicator of disease progression. BMI reduction rate has been inversely associated with survival time among ALS patients (Kamel et al., 2008; Shimizu et al., 2012). The median BMI reduction rate was 2.5 BMI units per year (interquartile range: 1.3–3.8), and was significantly correlated with survival length ( $p < 0.0001$ ) (Shimizu et al., 2012). A U-shaped relationship between BMI and mortality was reported, with the highest survival at 30–35 kg/m (O'Reilly et al., 2013). The adjusted HR for the linear association between BMI and survival was 0.860 (95% CI: 0.80–0.93,  $P = 0.0001$ ) (Paganoni et al., 2011). After adjustment, there was a 30% increase in risk of death for a 5% decrease from usual weight at time of diagnosis ( $RR = 1.30$ ; 95% CI: 1.08–1.56). During follow-up, the adjusted mortality risk identified was increased by 34% (95% CI: 18–51%) for each 5% decrease in usual weight and increased by 24% (95% CI: 13–36%) for each unit decrease in usual BMI ( $p < 0.0001$ ).

### 3.2.7. Nutritional status

Malnutrition during the disease course has been related to shorter survival ( $p = 0.01$ ), and fat mass associated with better outcomes ( $RR = 0.90$  for each 2.5 kg of fat mass) (Marin et al., 2011b), high serum levels of triglyceride is significantly associated with a longer survival in ALS patients (Huang et al., 2015). A recent study also found that severe vitamin D deficiency accelerated the rate of decline by 4 fold, and was associated with a shorter life expectancy (Camu et al., 2013).

### 3.2.8. Comorbidity

Comorbidity would be expected adversely to affect the disease course of ALS. However, an early report showed that an abnormally elevated LDL/HDL ratio significantly increased survival time by more than 12 months for ALS patients (Dupuis et al., 2008). This was further supported by a univariate analysis showing that a higher LDL/HDL ratio was correlated with increased survival ( $HR = 0.9$ ,  $p = 0.04$ ), after adjusting for possible confounding such as age at onset, onset site and FVC (Sutedja et al., 2011). Comorbidities that were investigated in four subsequent studies included cardiovascular disease, dementia, Parkinson's disease, and depressive symptoms (Paganoni et al., 2011; Chio et al., 2009b; Korner et al., 2013; Dedic et al., 2012): none were found to significantly influence ALS disease course or the survival rate of patients (Korner et al., 2013).

### 3.2.9. Smoking

Smoking adversely affects survival time in patients with ALS. Compared with never smokers, the mortality RR (95% CI) was 1.45 (1.03–2.03) for heavy smokers (Alonso et al., 2010a,b). Current smoking was also independently associated with shorter survival time ( $HR = 1.51$ , 95% CI: 1.07, 2.15) (de Jong et al., 2012). In addition, the shortened survival time associated with smoking was more significant for women than for men. Compared with never smokers, the mortality RR (95% CI) for ever smokers was 1.31 (1.04–1.65) for women and 0.90 (0.72–1.11) for men, and the risk was particularly elevated for women who were heavy smokers ( $HR = 1.94$ , 95% CI: 1.24–3.06) (Alonso et al., 2010a,b).

### 3.2.10. Heavy metals

The survival interval from diagnosis to death showed a weak inverse association with blood lead level ( $HR = 0.9$ , 95% CI: 0.8–1.0) and a stronger inverse association with patella lead (0.5, 95% CI: 0.2–1.0) and tibia lead (0.3, 95% CI: 0.1–0.7) (Kamel et al., 2008). Of course, exposure to lead is not beneficial. These results might

indicate that the individuals with ALS caused by lead had longer survival times than did other types of ALS presentation.

### 3.2.11. ALS among veterans

The median survival time for veterans with ALS from symptom onset was 4.7 years (3.3 years from diagnosis). Veterans with ALS who had been deployed in Vietnam had significantly shortened survival time [HR = 1.73 (95% CI: 1.36–2.19)] (Pastula et al., 2009).

### 3.2.12. Health care

Improved survival time among ALS cases has also been reported over the last two decades in the USA, Japan and Italy (Testa et al., 2004; Czaplinski et al., 2006; Kihira et al., 2008), a trend that might be related to improved health care (Gil et al., 2009). A retrospective study from England showed that independent of any intervention, the median survival from diagnosis was 19 months, for patients who attended the multidisciplinary clinic, compared to 11 months, for those attending the general neurology clinic (HR = 0.51, 95% CI: 0.41–0.64) (Aridegbe et al., 2013). A retrospective study from France also showed that more aggressive use of non-invasive ventilation apparatus significantly improved survival from 2002 to 2009 (Gordon et al., 2012). However, the effect of health care improvement on ALS survival was not observed in Scottish data (Forbes et al., 2004).

### 3.2.13. Uric acid

A meta-analysis revealed that serum uric acid level was significantly ( $p < 0.0001$ ) lower in ALS patients, particularly in cases with bulbar onset and longer disease duration (Abraham and Drory, 2014; Oh et al., 2015).

## 3.3. Genetic factors

### 3.3.1. ApoE

Five observational studies with the association between ApoE genotype and ALS progression could be identified. Two studies showed a significantly shorter survival time among ApoE  $\epsilon 4$  carriers (Drory et al., 2001; Moulard et al., 1996). The third one showed reduced survival time among  $\epsilon 4$  carriers with ALS, which was not statistically significant (Jawaid et al., 2011). The fourth and fifth studies showed no correlation between ApoE genotype and survival time (Lacomblez et al., 2002; Chio et al., 2016), but ApoE plasma levels were correlated with the rate of disease deterioration and survival time, with a relative risk of 0.647 (95% CI: 0.465–0.901;  $p = 0.01$ ) for decreased survival time (Lacomblez et al., 2002), and the presence of an ApoE  $\epsilon 2$  allele significantly increased the risk of FTD (odds ratio, 2.61; 95% CI, 1.14–6.10;  $P = 0.03$ ) (Chio et al., 2016).

### 3.3.2. Other genetic factors potentially associated with ALS progression

The minor allele carrier status of rs12608932, a common variant located within an intron of *UNC13A* gene on chromosome 19p13.3, was strongly associated with approximately 1-year reduced survival of ALS patients (Chio et al., 2013a,b). A significant effect of 'long' polyalanine repeat alleles of NIPA1 was identified in a large European genomic study (van Es et al., 2009). In patients carrying 'long' alleles, median survival was 3 months shorter than patients with 'normal' genotypes, and the onset of symptoms occurred 3.6 years earlier. Common variants (rs9897526, rs34424835, and rs850713) and haplotypes of *GRN* (the gene encoding progranulin) were significantly associated with a higher mortality after onset of ALS (HR = 2.5) (Slegers et al., 2008). Lower *SMN2* copy numbers and lower levels of estimated SMN protein (HR = 1.3, 95% CI: 1.1–1.6,  $p = 0.03$ ) were associated with an increased mortality rate in ALS patients (Veldink et al., 2005a). Survival analysis showed that

there was a lower survival probability in those with the risk allele (C allele: CC and CT genotypes) of the *ZNF512B* gene (log-rank test,  $P < 0.01$ ), independent of other prognostic factors in ALS (Tetsuka et al., 2013).

## 4. Discussion

ALS is a multifactorial neurodegenerative condition associated with modifiable environmental and unmodifiable genetic factors. Its rapid progression is mainly affected by un-modifiable risk factors, such as age at onset and disease type. Therefore, the discussion below seeks primarily to establish how likely it is for the risk factors for ALS onset identified in our analyses to be causal, as systematic review per se cannot be used to determine causality between risk factors and ALS.

### 4.1. Exposure to heavy metals

Exposure to heavy metals has been shown to increase the risk of ALS (Sutedja et al., 2009a,b; Johnson and Atchison, 2009; Ahmed and Wicklund, 2011; Vinceti et al., 2012; Iwami et al., 1994; Mitchell, 1987; Mitchell et al., 1991; Valentine et al., 2005; Trumbull and Beckman, 2009; Bowman et al., 2011; Caban-Holt et al., 2005). The most widely studied heavy metals in ALS patients are lead and mercury, both of which have been shown to be neuron toxicants in *in vitro* or *in vivo* studies, and to have accumulated in neural tissues (Kurlander and Patten, 1979). The modulation of normal SOD1 function by heavy metals is plausible because a causative link has been established between ALS and mutations in the SOD1 gene, from which the zinc/copper regulated superoxide degradation enzyme is synthesized. In addition, exposure to zinc either hastened or delayed the time of onset of ALS symptoms in an ALS animal model (Groeneveld et al., 2003; Ermilova et al., 2005). Further study is needed to clarify the role of zinc in the pathogenesis of ALS. A relationship between environmental selenium and ALS incidence has been reported (Vinceti et al., 2010). As the associations between ALS and zinc and ALS and selenium were largely inconclusive, the discussion will be focused on two heavy metals, lead and mercury.

#### 4.1.1. Lead

A fuller discussion of the evidence on the association between lead and ALS has been published by (Wang et al., 2014b). Speculation about the association between exposure to lead and ALS began about 5 decades ago, when a series of ALS cases with antecedent lead exposure was reported (Oh et al., 2007; Livesley and Sissons, 1968). Since then, over a dozen retrospective case-control studies have been conducted. Most studies clearly showed that occupational exposure to lead was associated with a higher risk of ALS (Kamel et al., 2005; Scarpa et al., 1988), although some studies failed to observe a statistically significant association (Gresham et al., 1992; Pierce-Ruhland and Patten, 1981; Qureshi et al., 2006). The overall estimate of the risk of ALS effect due to previous exposure to lead in the meta-analysis conducted in this study was statistically significant. This association was supported by the following dose-response studies. One study showed an incidence gradient in the vicinity of a lead smelting factory in a county of the state of Missouri (Turabelidze et al., 2008). Kamel and his colleagues explored this association, with ALS cases and controls identified in Boston, using various lead measurement technologies (Fang et al., 2009; Kamel et al., 2002, 2005). A linear response relationship between lead exposure and ALS prevalence was confirmed in one case-control study (Fang et al., 2010), which was consistent with previous case-control studies (Fang et al., 2009; Kamel et al., 2002; Campbell et al., 1970). A major challenge in exploring the association between lead exposure and ALS is in

the determination of historical exposure levels to lead. Since the lead levels in blood or other body liquids may not represent previous lead exposure, it is not surprising that some studies did not observe a difference in lead exposure between cases and controls (Kamel et al., 2005).

Paradoxically, the blood or bone levels of lead were positively associated with survival in a case-control study and in an animal study (Barbeito et al., 2010; Kamel et al., 2008). One possible explanation for this observation was that lead exposure might increase the production of antioxidants or stimulate VEGF (vascular endothelial growth factor) expression (Johnson and Atchison, 2009; Barbeito et al., 2010).

#### 4.1.2. Mercury

Neurotoxic effects of mercury in humans and animals are well documented. *In vitro* experiments have shown that mercury damages the axons of neuron cells, a typical pathological change of ALS neuronal degeneration. Insufficient studies relating to mercury risk for ALS onset were identified for a meta-analysis. Although there are case reports of ALS associated with previous exposure to mercury, the relationship between the risk of ALS and exposure to mercury is yet to be established in analytical epidemiological studies. *In vivo* experiments have shown that mercury deposits in the nervous system and damages the axons of motor neurons, a pathological change that is typical of ALS neuronal degeneration (Pamphlett and Png, 1998). Chronic exposure to methyl-mercury induced early onset of hind limb weakness in a transgenic ALS mouse model, indicating that methyl-mercury promotes the progression of ALS (Callaghan et al., 2011), and may increase the risk of onset of ALS in humans.

#### 4.2. Previous exposure to pesticides

An association between previous exposure to pesticides and ALS was identified in the systematic review and meta-analysis of observational studies conducted here, consistent with three recently published analyses (Kamel et al., 2012; Malek et al., 2012; Kang et al., 2014), and reported similar risk estimates as ours. Evidence supporting the hypothesis that exposure to pesticides as a causal factor for ALS is discussed below.

##### 4.2.1. Indirect evidence from genetic studies

Genetic studies have suggested that previous exposure to pesticides may be a strong risk factor for ALS (Morahan et al., 2007). The different variants of PON1 (paraoxonase/arylesterase 1), an enzyme which detoxifies organophosphate pesticides, have been associated with the occurrence of sporadic ALS in some publications (Saeed et al., 2006). A meta-analysis of the overall association between PON1 variant and risk of ALS was a minor increase [OR = 1.10 (1.01–1.20)] with borderline statistical significance (Wills et al., 2009), although the statistical significance was diminished if the included studies were adjusted for Hardy–Weinberg equilibrium ( $p > 0.05$ ). However a causal association between PON1 and ALS should not be simply ruled out, because not all PON1 gene variants have been investigated. The sample size in each study might have been too small to demonstrate a significant association. A recent Italian study identified a higher frequency of PON1 gene mutations among ALS cases, compared to controls (Ticozzi et al., 2010); this article has provided the strongest evidence of an association between PON1 and ALS.

##### 4.2.2. Chronic toxicity of organophosphorus compounds

A link between previous exposure to pesticides and ALS is supported by symptoms of chronic toxicity resulting from exposure to organophosphorus compounds (OPCs). OPCs have been used as pesticides, insecticides, fertilizers, and nerve agents

in warfare for more than a century. OPCs can cause several types of neurological disorders in humans, depending on the exposure profile, amount, duration, and pathways (Jokanovic and Kosanovic, 2010). Organophosphate-induced delayed polyneuropathy (OPIDP) is thought to be similar to ALS. Investigators described an outbreak of OPIDP due to the contamination of soil by OPCs, which occurred in 1942 on a farm in Italy (Tosi et al., 1994). The patients in this outbreak were originally diagnosed with polyneuritis, with symptoms described as progressive, beginning with the limbs, but sensory nerves were not affected. The clinical manifestations would be consistent with current diagnostic criteria for ALS (Tosi et al., 1994). Similar syndromes have also been reported in some developing countries. A report from China described 143 cases with delayed dyspnea caused by pesticide poisoning, some of which resembled ALS (Zhang, 1991).

The most convincing epidemiological evidence of the association between exposure to pesticides and risk of ALS was reported in a prospective study, in which the mortality due to ALS in a pesticide factory was over 3-fold greater than expected ( $P < 0.05$ ) (Burns et al., 2001). In addition, the increased incidence of mortality from ALS among soccer players, football players, baseball players, and among veterans could be partially explained by exposure to pesticides or other agricultural chemicals (Chio et al., 2009a; Vanacore et al., 2006; Belli and Vanacore, 2005; Wicks et al., 2007; Weisskopf et al., 2005a,b).

##### 4.2.3. Evidence from case reports

ALS cases following exposure to agricultural chemicals have also been widely reported (Pall et al., 1987; Fonseca et al., 1993; Ahdab et al., 2011). The most relevant ALS case supporting a causal association between ALS and exposure to pesticides was reported (Doi et al., 2006). The disease progression in this case was modulated by the presence and absence of the pesticide.

Taken together, these observations favour the hypothesis that exposure to pesticides might be causally associated with ALS. This association could partially explain why professional soccer players (Chio et al., 2009a), foot players (Abel, 2007), and veterans who have ever participated in wars, but not professional basketball players or cyclists, have a higher risk of developing ALS.

##### 4.3. Previous trauma

A history of trauma was identified as a risk factor from the first reported cases of ALS (Alpers and Farmer, 1949). It is important to determine how one or several repetitive local injuries could initiate ALS, a disease that progresses rapidly from the onset site to other parts of the body, for which hypotheses have been proposed (Gargiulo-Monachelli et al., 2012). The damage could spread, even it may spread further to the opposite motor cortex, the brainstem, and the spine. Two small-scale pathological studies supported the association between brain injury and ALS (McKee et al., 2009, 2013). The meta-analysis conducted in this study also identified a strong association between previous traumas and ALS. Further meta-analysis also revealed that ALS was associated with old trauma, which occurred at least 5 years prior to a diagnosis of ALS. This association supports the view that trauma may be a causal factor, because old trauma is unlikely to occur as a consequence of ALS. Therefore, although two recent studies argued that the traumas were the consequence instead of the cause of ALS (Beghi et al., 2010; Turner et al., 2010), previous trauma should still be considered a potential risk factor for ALS (Pupillo et al., 2012).

If previous trauma is a risk factor for ALS, then some risk factors associated with ALS – professional sports, lower BMI, lower educations, and strenuous work – may have been confounded by previous trauma. All of these potential associations are discussed further below.

#### 4.3.1. Physical activity and participation in organized sports

Participating in sports or physical activities has been associated with ALS in some, but not all, early studies. If professional sports were excluded, the overall risk estimate from our meta-analysis showed that participating in sports was not significantly associated with an increased risk of ALS. This result is consistent with a comprehensive study from Veldink's group (Veldink et al., 2005b). However, we did find that participating in organized or professional sports was significantly associated with ALS. These different estimates could explain some conflicting observations in literature about the association between sports and ALS (Longstreth et al., 1998; Veldink et al., 2005b; Qureshi et al., 2006; Valenti et al., 2005). Many studies did not distinguish general physical activity and sports from the professional organized sports (Okamoto et al., 2009b), and summarized the results together (Hamidou et al., 2014). The possible explanations for the difference between professional and recreational sports are that professional athletes have a higher risk of getting trauma/injury (Harwood et al., 2009), and exposing to pesticides (Su et al., 2016).

#### 4.3.2. Blue collar occupation and lower education

As individuals involved in blue collar occupations often possess lower education levels, thus these two risk factors are correlated. Early epidemiological studies, especially those conducted in Italy, found that workers having strenuous occupations had a higher risk of developing ALS (Chancellor et al., 1993; Gunnarsson et al., 1991; Buckley et al., 1983). These occupations required repetitive motions or heavy labour, such as professional sports, occupations related to primary and secondary industries, including construction and mining. Repetitive work itself may not be able to cause motor neuron degeneration which occurs in the brain motor cortex, the spine, and the brainstem through common pathological change. Many potential risk factors, such as exposure to pesticides, mechanical injury, exposure to heavy metals, and smoking have been associated with ALS, but actually these potential risk factors are highly correlated with blue collar occupations and could be good surrogate variables for blue collar occupations.

#### 4.3.3. High fitness level

Three publications reported an association between having a high fitness level and ALS. The first article showed an inverse association between BMI and the incidence of ALS among college varsity athletes (Scarmeas et al., 2002). Two subsequent studies confirmed this observation, showing that the incidence of ALS was less common in patients with cardiovascular disease (CVD) than in non-CVD patients (O'Reilly et al., 2013; Sutedja et al., 2011). These two studies concluded that comparing to control population, the BMI value was significantly lower in ALS patients prior to the onset of disease, along with significant higher physical strength. However, these three studies could not rule out confounding effects from head and spinal trauma/injuries. The individuals with lower BMI in these studies were army or professional sport participants. They are physically active, and were more likely to be injured than individuals with higher BMI in general population. In addition, a higher BMI has been consistently demonstrated being a protective factor against ALS progression (Paganoni et al., 2011), the actual involved mechanism is unknown.

### 4.4. Lifestyle

#### 4.4.1. Smoking

Early studies produced controversial results about the association between smoking and ALS (Kamel et al., 1999; Fang et al., 2006; Qureshi et al., 2006; Nelson et al., 2000). In the present

analysis, we found that the association between smoking and ALS was significant in females, not in males. Since a high proportion of bulbar onset ALS cases have occurred in females, smoking may be a risk factor for bulbar ALS, or may promote early onset in females (Korczyn and Drory, 2010). A meta-analysis of evidence from cohort studies suggested that smoking was a causal risk factor for ALS in females, with former smokers found to be at higher risk of ALS (Wang et al., 2011a,b). Since males tend to be more frequently exposed to other potential ALS risk factors, such as pesticides or organic solvents during their working life, the lack of associations in males might have been confounded by occupation. More population-based studies designed to study ALS aetiology are required, as most of the studies quoted in this project were not designed specifically for that (Wang et al., 2011a,b; Gallo et al., 2009). Even though smoking is not a strong risk factor for ALS, patients should be encouraged to quit smoking because smoking has been associated with higher incidences of other chronic diseases, including cardiovascular disease and cancer. One potential mechanism by which smoking could affect the risk of ALS is the possible impairment of ER (endoplasmic reticulum) and mitochondria (Federico et al., 2012) by the oxidization products from smoking (Alonso et al., 2010a,b). The contamination of tobacco by heavy metals and pesticides could be another possible explanation for the association between ALS and smoking (Weisskopf et al., 2010).

#### 4.4.2. Alcohol and coffee consumption

Light to moderate alcohol consumption has been reported to be inversely associated with the incidence of dementia and Alzheimer's disease (Davignus et al., 2011). An observational study recently found that current alcohol consumption was associated with a reduced risk of ALS (de Jong et al., 2012). Our preliminary meta-analysis does not support an association between alcohol consumption and ALS risk. However, this association might not indicate a protective effect of alcohol *per se*, because a risk factor must exert its effect prior to ALS onset, rather than contemporaneously. The same argument also could apply to the inverse association between coffee consumption and ALS (Beghi et al., 2011).

#### 4.4.3. Vitamin E intake

It has been suggested that oxidative stress resulting from reactive oxygen species (ROS) is associated with ALS onset. In comparison to controls, oxidative products in plasma and SOD activity in erythrocytes were elevated in ALS patients (Bonnetfont-Rousselot et al., 2000; Oteiza et al., 1997). Increase in lipid peroxidation in spinal cord was observed prior to the onset of ultrastructure or clinical changes in SOD1 transgenic mouse model of familial ALS (Hall et al., 1998). The greatest intensity of motor neuronal injury due to lipid peroxidation was observed in ALS patients in the active phase of disease progression (Hall et al., 1998). Oxidant treatment of cultured motor neurons has caused a dose-dependent increase in apoptosis (Kaal et al., 1998). In addition, an epidemiological case-control study found that a higher intake of food rich in antioxidants, such as fruit and vegetables, might play a protective role against the development of ALS (Okamoto et al., 2009a). Therefore, intake of vitamin E, an antioxidant, could be a protective factor against ALS. However, supplementary vitamin E given to ALS patients was ineffective in arresting disease progression. After 12 months of treatment, supplementary vitamin E had no effect on disease progression or survival time (Desnuelle et al., 2001; Graf et al., 2005; Galbussera et al., 2006), indicating that progression of the disease was not reversible by vitamin E.

#### 4.5. Electric shock

Early epidemiological studies showed that occupational exposure to electromagnetic fields was positively associated with ALS (Li and Sung, 2003), and a synthetic OR, based on a meta-analysis, was significantly elevated (Zhou et al., 2012). However, animal experiments failed to establish an association (Johnson and Atchison, 2009; Poullietier de Gannes et al., 2009), raising the possibility of confounding by other risk factors in some human population studies. A large prospective study in Switzerland found that distance from the magnetic field resulting from hydro power-lines was not associated with an altered risk of developing ALS (Huss et al., 2009). Since our meta-analysis showed an association of ALS with a history of electric shock, the association of occupational exposure to electromagnetic fields with ALS could be confounded by a history of electric shock. Many ALS cases have been diagnosed following electric shock (Al-Ajmi et al., 2012; Sirdofsky et al., 1991; Rose, 1994). These case reports form the strongest evidence of a causative association between electric shock and ALS. A review indicated that the evidence linking electrical occupations to an increased risk for ALS was remarkably consistent, but that the evidence of an association with measured magnetic field levels was weak (Consaes et al., 2012). In agreement with our conclusion, a recent meta-analysis of observational studies did not support an association between extremely low frequency magnetic fields and ALS (Vergara et al., 2013). Based on these observations, we hypothesize that electric shock is the one of confounding factors, although the mechanisms by which electric shock could cause ALS are not known, and a low quality systematic review failed to reveal any association with ALS (Abhinav et al., 2007).

#### 4.6. Intake of beta-N-methylamino-L-alanine (BMAA)

The incidence of ALS-PDC (ALS-Parkinson-Dementia-Complex) syndrome, in Guam and surrounding Pacific islands, was found to be up to 50–100 fold greater than the average incidence of ALS worldwide during the 1940s to the 1960s. It did decline to rates similar to those reported in other regions after 1980, before its cause was established (Stone, 1993). The excess cases of ALS-PDC in Guam and surrounding Pacific islands were first associated with the food toxicant BMAA in 1987, after partial replication of both clinical and pathological changes typical of ALS-PDC resulting from high doses of BMAA in monkeys (Spencer et al., 1987a,b; Spencer, 1987). BMAA is a neurotoxic amino acid, which can be produced by most aquatic cyanobacteria. BMAA neurotoxicity occurs via the activation of glutamate metabotropic receptors (mGluR5 and mGluR1) and the induction of oxidative stress (Banack et al., 2010). This hypothesis was initially overlooked, possibly due to negative follow-up studies in mice (Perry et al., 1989; Cruz-Aguado et al., 2006), and the discovery of less than sufficient residual amounts of BMAA in the cycad seed flour, which was consumed by the local residents in Guam, after conventional washing processes (Duncan et al., 1990). However, this unproven hypothesis was revived 15 years later by Cox et al. (2009), after bio-magnification of BMAA was observed in foxes, which used to be consumed by the Chamorro population. The presence of BMAA in brain tissue was also reported in ALS-PDC cases (Murch et al., 2004). Clinical and pathological changes mimicking ALS in rats has resulted from exposure to BMAA (de Munck et al., 2013). Despite the return of the BMAA hypothesis, causal association has still not been established in humans. The disease term ALS-PDC was excluded from our selection of ALS disease terms since we were not sure that the form of ALS seen in the Chamorro people was same as sporadic ALS in other regions of the world. It was believed that the environmental

risk factor associated with ALS-PDC was unique to Guam and the surrounding areas when this work was initiated. Since the present study was designed to synthesize epidemiological studies, most studies related to BMAA would have been excluded even if ALS-PDC term was included in the search.

The high incidence of ALS in the Pacific islands has declined over the last four decades, which may be due to lifestyle and dietary changes, and is now close to the incidence reported in other regions. Whether this food toxicant theory is true or not, the story of the temporary ALS epidemic in Guam and other Pacific islands unequivocally indicates that environmental factors are associated with ALS. Similar temporal fluctuations in ALS incidence, but to a much lower degree, has also been observed in many other areas worldwide (Gordon et al., 2011a; Doi et al., 2010; Alonso et al., 2011; Georgouloupoulou et al., 2011), further underscoring the role of environmental factors in the pathogenesis of sporadic ALS.

#### 4.7. Military service

Military service may not be an independent risk factor for ALS because the number of wars participated is linearly associated with ALS. The organic phosphorus compounds present in explosives and war associated trauma/injuries (Bergman et al., 2015) or exposure to chemicals (Beard and Kamel, 2015) might be the underlying risk factors for ALS in military personnel or veterans.

#### 4.8. Viral infection

Because ALS specifically affects voluntary skeletal muscles, an immune response elicited by viral infection might cause paralysis in a tissue (motor neuron) specific manner, leading to the speculation that viral infection may be a risk factor for ALS. Such speculation is consistent with the abnormal immune response observed in ALS patients (Rentzos et al., 2011). The hypothesis of viral infection and subsequent immune response could explain the specificity of ALS affected tissues. Unfortunately, convincing epidemiological evidence supporting this association has not been found.

### 5. Possible biological interpretations of risk factors associated with ALS

Risk factors for development and progression of ALS may be considered with the context of the process of prion-like propagated protein misfolding, which is relevant not only to ALS, but to other neurodegenerative diseases such as Alzheimer's and Parkinson's disease (Guest et al., 2011). Two proteins participate in this prion-like propagation of protein misfolding in ALS: CuZn superoxide dismutase (SOD1) (Grad et al., 2011; Smethurst et al., 2014) and TDP43 (Nonaka et al., 2013). Both proteins are known to become misfolded and aggregated under various conditions of cell stress. Ataxin-2 may participate in the expression of 'seeding' proteins or co-factors in their generation (Wang et al., 2014a). Exposure to heavy metals such as lead and mercury, as well as to pesticides and solvents, is known to post-translationally modify or oxidize proteins *in vivo* (Stohs and Bagchi, 1995; Braconi et al., 2011) and could be associated with formation of a productive nidus or 'seed' which can propagate its misfold intracellularly and intercellularly. Smoking-associated formaldehyde exposure can also post-translationally modify/cross-link lysine, arginine and tryptophan in proteins (Edrissi et al., 2013; Reisner and Lundblad, 2009), perhaps including those proteins that can provide a productive seeding template for ALS. Electric shock could heat-denature proteins (Tsong and Su, 1999) to potentially form a productive misfolded protein seed that could propagate to non-injured regions. Military service may also have been thought to

be associated with exposure to BMAA (Cox et al., 2009), which can disturb conformation of proteins though incorporation of BMAA as a natural but non-protein amino acid (Glover et al., 2014), additional to the association with trauma and explosive compounds. Heavy work and strenuous athletic activity are associated with the generation of reactive oxygen species (Parker et al., 2014), which may change protein conformation via oxidative modification of the free-radical defence enzyme SOD1. Viral infection can alter protein translation and chaperone availability of proteins in the nervous system. Once a propagated protein misfolding process has been seeded, different factors may impact on the rate of progression of disease, including the availability of fat reserves to tolerate decrease nutritional intake, vitamin D deficiency, comorbidities, and other factors that are not related to the initial genesis of the original template for misfolding.

## 6. Conclusions

ALS is a multifactorial neurodegenerative condition that affects primarily voluntary muscles. No single risk factor has been identified that explains a significant proportion of total ALS cases. Mutations in the two most common monogenic risk factors, SOD1 and C9ORF72, could explain about 25% and 35%, respectively, of familial ALS cases. All the monogenic mutations, in over 30 genes, could explain about 10% all ALS cases (Chio et al., 2012). For all environmental or other genetic risk factors identified in the present study, each risk factor was associated with only a small proportion of total ALS cases.

One genetic factor (*ATXN2*) and five environmental factors (previous exposure to heavy metals including lead, previous exposure to pesticides, a history of physical trauma/injury, a history of electronic shock, and previous exposure to organic solvents) have been identified with relatively strong associations with the onset of ALS in this study, supporting multi-factorial and multi-pathway etiology of ALS. Further studies are required to verify these findings. With respect to the progression of ALS, age and disease type were the two most important risk factors determining the survival of ALS patients. Modifiable risk factors, such as nutrition status and BMI level, have also been found to modify the survival time of individuals with ALS. Genetic risk factors may also modify the progression of ALS. Although the current study has identified a number of potential risk factors associated with the onset or progression of ALS, further work is required to establish causal associations. In addition, as the number of studies included in most meta-analyses in this study was small, caution should be used when interpreting the findings.

## Conflict of interest

The authors declare that there are no conflicts of interest.

## Acknowledgements

Financial Support for this project was provided by the Public Health Agency of Canada in association with Neurological Health Charities Canada through a contribution agreement administered through the University of Ottawa. N. Cashman holds a Canada Research Chair in Neurodegeneration and Protein Misfolding Diseases at the University of British Columbia; J. Little holds a Canada Research Chair in Human Genome Epidemiology at the University of Ottawa; D. Krewski holds the McLaughlin Chair in Risk Science at the University of Ottawa. The authors acknowledge the expert opinions expressed by Dr. Hannah Briemberg on an earlier draft of this manuscript.

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.neuro.2016.06.015>.

## References

- ALSGEN Consortium, Ahmeti, K.B., Ajroud-Driss, S., Al-Chalabi, A., Andersen, P.M., Armstrong, J., et al., 2013. Age of onset of amyotrophic lateral sclerosis is modulated by a locus on 1p34.1. *Neurobiol. Aging* 34, 357 (e7357.19).
- Abel, O., Powell, J.F., Andersen, P.M., Al-Chalabi, A., 2012. ALSod: a user-friendly online bioinformatics tool for amyotrophic lateral sclerosis genetics. *Hum. Mutat.* 33, 1345–1351.
- Abel, E.L., 2007. Football increases the risk for Lou Gehrig's disease, amyotrophic lateral sclerosis. *Percept. Mot. Skills* 104, 1251–1254.
- Abhinav, K., Al-Chalabi, A., Hortobagyi, T., Leigh, P.N., 2007. Electrical injury and amyotrophic lateral sclerosis: a systematic review of the literature. *J. Neurol. Neurosurg. Psychiatry* 78, 450–453.
- Abraham, A., Drory, V.E., 2014. Influence of serum uric acid levels on prognosis and survival in amyotrophic lateral sclerosis: a meta-analysis. *J. Neurol.* 261, 1133–1138.
- Aggarwal, A., Nicholson, G., 2005. Age dependent penetrance of three different superoxide dismutase 1 (sod 1) mutations. *Int. J. Neurosci.* 115, 1119–1130.
- Ahdab, R., Ayache, S.S., Maltonti, F., Brugieres, P., Lefaucheur, J.P., 2011. Motor neuron disorder with tongue spasms due to pyrethroid insecticide toxicity. *Neurology* 76, 196–197.
- Ahmed, A., Wicklund, M.P., 2011. Amyotrophic lateral sclerosis: what role does environment play. *Neurol. Clin.* 29, 689–711.
- Al-Ajmi, A., Rousseff, R.T., Khuraibet, A.J., 2012. Clinically definite ALS presenting weeks after mild electric injury: causality or coincidence? *Neurol. Sci.*
- Al-Chalabi, A., Fang, F., Hanby, M.F., Leigh, P.N., Shaw, C.E., Ye, W., et al., 2010. An estimate of amyotrophic lateral sclerosis heritability using twin data. *J. Neuro. Neurosurg. Psychiatry* 1324–1326 PMC2988617 (December 01).
- Alonso, A., Logroscino, G., Jick, S.S., Hernan, M.A., 2009. Incidence and lifetime risk of motor neuron disease in the United Kingdom: a population-based study. *Eur. J. Neurol.* 16, 745–751.
- Alonso, A., Logroscino, G., Hernan, M.A., 2010a. Smoking and the risk of amyotrophic lateral sclerosis: a systematic review and meta-analysis. *J. Neurol. Neurosurg. Psychiatry* 81, 1249–1252.
- Alonso, A., Logroscino, G., Jick, S., Hernan, M., 2010b. Association of smoking with amyotrophic lateral sclerosis risk and survival in men and women: a prospective study. *BMC Neurol.* 10.
- Alonso, V., Villaverde-Hueso, A., Hens, M.J., Morales-Piga, A., Abaitua, I., de la Paz, M. P., 2011. Increase in motor neuron disease mortality in Spain: temporal and geographical analysis (1990–2005). *Amyotroph. Lateral Scler.* 12, 192–198.
- Alpers, B.J., Farmer, R.A., 1949. Role of repeated trauma by pneumatic drill in production of amyotrophic lateral sclerosis. *Arch. Neurol. Psychiatry* 62, 178–182.
- Alsod, U., ALS ONLINE GENETICS DATABASE, World Federation of Neurology and European Network to Cure ALS. Jan. 2014; 2014.
- The Amyotrophic Lateral Sclerosis Functional Rating Scale, 1996. Assessment of activities of daily living in patients with amyotrophic lateral sclerosis. The ALS CNTF treatment study (ACTS) phase I–II study group. *Arch. Neurol.* 53, 141–147.
- Aridege, T., Kandler, R., Walters, S.J., Walsh, T., Shaw, P.J., McDermott, C.J., 2013. The natural history of motor neuron disease: assessing the impact of specialist care. *Amyotroph. Lateral Scler. Frontotemporal. Degener.* 14, 13–19.
- Armon, C., 2009. Smoking may be considered an established risk factor for sporadic ALS. *Neurology* 73, 1693–1698.
- Banack, S.A., Caller, T.A., Stommel, E.W., 2010. The cyanobacteria derived toxin Beta-N-methylamino-L-alanine and amyotrophic lateral sclerosis. *Toxins (Basel)* 2, 2837–2850.
- Barbeito, A.G., Martinez-Palma, L., Vargas, M.R., Pehar, M., Manay, N., Beckman, J.S., et al., 2010. Lead exposure stimulates VEGF expression in the spinal cord and extends survival in a mouse model of ALS. *Neurobiol. Dis.* 37, 574–580.
- Barth, S., Kang, H., Bullman, T., Wallin, M., 2009. Neurological mortality among US veterans of the Persian Gulf War: 13-year follow-up. *Am. J. Ind. Med.* 52, 663–670.
- Beard, J.D., Kamel, F., 2015. Military service, deployments, and exposures in relation to amyotrophic lateral sclerosis etiology and survival. *Epidemiol. Rev.* 37, 55–70.
- Beghi, E., Logroscino, G., Chio, A., Hardiman, O., Millul, A., Mitchell, D., et al., 2010. Amyotrophic lateral sclerosis, physical exercise, trauma and sports: results of a population-based pilot case-control study. *Amyotroph. Lateral Scler.* 11, 289–292.
- Beghi, E., Pupillo, E., Messina, P., Giussani, G., Chio, A., Zoccollella, S., et al., 2011. Coffee and amyotrophic lateral sclerosis: a possible preventive role. *Am. J. Epidemiol.* 174, 1002–1008.
- Belbasis, L., Bellou, V., Evangelou, E., 2016. Environmental risk factors and amyotrophic lateral sclerosis: an umbrella review and critical assessment of current evidence from systematic reviews and meta-analyses of observational studies. *Neuroepidemiology* 46, 96–105.
- Belli, S., Vanacore, N., 2005. Proportionate mortality of Italian soccer players: is amyotrophic lateral sclerosis an occupational disease. *Eur. J. Epidemiol.* 20, 237–242.

- Berg, L., de Jong, S., Huisman, M., Van der Kooi, A., De Visser, M., Schelhaas, H., et al., 2011. Smoking, alcohol consumption and the risk of amyotrophic lateral sclerosis: a population-based study. *Neurology* 76, A115.
- Berger, M.M., Kopp, N., Vital, C., Redl, B., Aymard, M., Lina, B., 2000. Detection and cellular localization of enterovirus RNA sequences in spinal cord of patients with ALS. *Neurology* 54, 20–25.
- Bergman, B.P., Mackay, D.F., Pell, J.P., 2015. Motor neurone disease and military service: evidence from the Scottish Veterans Health Study. *Occup. Environ. Med.* 72, 877–879.
- Binazzi, A., Belli, S., Uccelli, R., Desiato, M.T., Talamasca, I.F., Antonini, G., et al., 2009. An exploratory case-control study on spinal and bulbar forms of amyotrophic lateral sclerosis in the province of Rome. *Amyotroph. Lateral Scler.* 10, 361–369.
- Bonnefont-Rousselot, D., Lacomblez, L., Jaudon, M., Lepage, S., Salachas, F., Bensimon, G., et al., 2000. Blood oxidative stress in amyotrophic lateral sclerosis. *J. Neurol. Sci.* 178, 57–62.
- Bowman, A.B., Kwakye, G.F., Hernandez, E.H., Aschner, M., 2011. Role of manganese in neurodegenerative diseases. *J. Trace Elem. Med. Biol.* 25, 191–203.
- Braconi, D., Bernardini, G., Santucci, A., 2011. Linking protein oxidation to environmental pollutants: redox proteomic approaches. *J. Proteomics* 74, 2324–2337.
- Brody, J.A., Grant, M.D., 2001. Age-associated diseases and conditions: implications for decreasing late life morbidity. *Aging (Milano)* 13, 64–67.
- Bryan, L., Kaye, W., Antao, V., Mehta, P., Muravov, O., Horton, D.K., 2016. Preliminary results of national amyotrophic lateral sclerosis (ALS) registry risk factor survey data. *PLoS One* 11, e0153683.
- Buckley, J., Warlow, C., Smith, P., Hilton-Jones, D., Irvine, S., Tew, J.R., 1983. Motor neuron disease in England and Wales, 1959–1979. *J. Neurol. Neurosurg. Psychiatry* 46, 197–205.
- Burns, C.J., Beard, K.K., Cartmill, J.B., 2001. Mortality in chemical workers potentially exposed to 2,4-dichlorophenoxyacetic acid (2,4-D) 1945–94: an update. *Occup. Environ. Med.* 58, 24–30.
- Byrne, S., Elamin, M., Bede, P., Shatunov, A., Walsh, C., Corr, B., et al., 2012. Cognitive and clinical characteristics of patients with amyotrophic lateral sclerosis carrying a C9orf72 repeat expansion: a population-based cohort study. *Lancet Neurol.* 11, 232–240.
- Caban-Holt, A., Mattingly, M., Cooper, G., Schmitt, F.A., 2005. Neurodegenerative memory disorders: a potential role of environmental toxins. *Neurol. Clin.* 23, 485–521.
- Callaghan, B., Feldman, D., Gruis, K., Feldman, E., 2011. The association of exposure to lead, mercury, and selenium and the development of amyotrophic lateral sclerosis and the epigenetic implications. *Neurodegenerative Dis.* 8, 1–8.
- Caller, T.A., Doolin, J.W., Haney, J.F., Murby, A.J., West, K.G., Farrar, H.E., et al., 2009. A cluster of amyotrophic lateral sclerosis in New Hampshire: a possible role for toxic cyanobacteria blooms. *Amyotroph. Lateral Scler. Off. Publ. World Fed. Neurol. Res. Group Motor Neuron Dis.* 10 (Suppl 2 (Jan 01)), 101–108.
- Caller, T.A., Field, N.C., Chipman, J.W., Shi, X., Harris, B.T., Stommel, E.W., 2012. Spatial clustering of amyotrophic lateral sclerosis and the potential role of BMAA. *Amyotroph. Lateral Scler.* 13, 25–32.
- Campbell, A.M., Williams, E.R., Barltrop, D., 1970. Motor neurone disease and exposure to lead. *J. Neurol. Neurosurg. Psychiatry* 33, 877–885.
- Camu, W., Tremblie, B., Plassot, C., Alphandery, S., Salsac, C., Pageot, N., et al., 2013. Vitamin D confers protection to motoneurons and is a prognostic factor of amyotrophic lateral sclerosis. *Neurobiol. Aging*.
- Cannon, J.R., Timothy Greenamyre, J., 2011. The role of environmental exposures in neurodegeneration and neurodegenerative diseases. *Toxicol. Sci.*
- Catala-Lopez, F., Suarez-Pinilla, M., Suarez-Pinilla, P., Valderas, J.M., Gomez-Beneyto, M., Martinez, S., et al., 2014. Inverse and direct cancer comorbidity in people with central nervous system disorders: a meta-analysis of cancer incidence in 577,013 participants of 50 observational studies. *Psychother. Psychosom.* 83, 89–105.
- Cermelli, C., Vinceti, M., Beretti, F., Pietrini, V., Nacci, G., Pietrosemoli, P., et al., 2003. Risk of sporadic amyotrophic lateral sclerosis associated with seropositivity for herpesviruses and echovirus-7. *Eur. J. Epidemiol.* 18, 123–127.
- Ceroni, M., Malaspina, A., Poloni, T.E., Alimonti, D., Rognoni, F., Habgood, J., et al., 1999. Clustering of ALS patients in central Italy due to the occurrence of the L84F SOD1 gene mutation. *Neurology* 53, 1064–1071.
- Chancellor, A.M., Slattery, J.M., Fraser, H., Warlow, C.P., 1993. Risk factors for motor neuron disease: a case-control study based on patients from the Scottish Motor Neuron Disease Register. *J. Neurol. Neurosurg. Psychiatry* 56, 1200–1206.
- Chen, Y., Huang, R., Chen, K., Song, W., Yang, Y., Zhao, B., et al., 2012. Association analysis of PON polymorphisms in sporadic ALS in a Chinese population. *Neurobiol. Aging* 33, 2949 (e12949, e3).
- Chen, Y., Li, S., Su, L., Sheng, J., Lv, W., Chen, G., et al., 2015. Association of progranulin polymorphism rs5848 with neurodegenerative diseases: a meta-analysis. *J. Neurol.* 262, 814–822.
- Chen, Y., Lin, Z., Chen, X., Cao, B., Wei, Q., Ou, R., et al., 2016. Large C9orf72 repeat expansions are seen in Chinese patients with sporadic amyotrophic lateral sclerosis. *Neurobiol. Aging* 38, 217 (e15217, e22).
- Chio, A., Calvo, A., Dossena, M., Ghiglione, P., Mutani, R., Mora, G., 2009a. ALS in Italian professional soccer players: the risk is still present and could be soccer-specific. *Amyotroph. Lateral Scler.* 10, 205–209.
- Chio, A., Calvo, A., Iardi, A., Cavallo, E., Moglia, C., Mutani, R., et al., 2009b. Lower serum lipid levels are related to respiratory impairment in patients with ALS. *Neurology* 73, 1681–1685.
- Chio, A., Logroscino, G., Hardiman, O., Swigler, R., Mitchell, D., Beghi, E., et al., 2009c. Prognostic factors in ALS: a critical review. *Amyotroph. Lateral Scler.* 10, 310–323.
- Chio, A., Calvo, A., Moglia, C., Mazzini, L., Mora, G., 2011. PARALS study group: phenotypic heterogeneity of amyotrophic lateral sclerosis: a population based study. *J. Neurol. Neurosurg. Psychiatry* 82, 740–746.
- Chio, A., Calvo, A., Mazzini, L., Cantello, R., Mora, G., Moglia, C., et al., 2012. Extensive genetics of ALS: a population-based study in Italy. *Neurology* 79, 1983–1989.
- Chio, A., Hammond, E.R., Mora, G., Bonito, V., Filippini, G., 2013a. Development and evaluation of a clinical staging system for amyotrophic lateral sclerosis. *J. Neurol. Neurosurg. Psychiatry*.
- Chio, A., Mora, G., Restagno, G., Brunetti, M., Ossola, I., Barberis, M., et al., 2013b. UNC13A influences survival in Italian amyotrophic lateral sclerosis patients: a population-based study. *Neurobiol. Aging* 34, 357 (e1357, e5).
- Chio, A., Brunetti, M., Barberis, M., Iazzolino, B., Montuschi, A., Iardi, A., et al., 2016. The role of APOE in the occurrence of frontotemporal dementia in amyotrophic lateral sclerosis. *JAMA Neurol.* 73, 425–430.
- Consales, C., Merla, C., Marino, C., Benassi, B., 2012. Electromagnetic fields, oxidative stress, and neurodegeneration. *Int. J. Cell Biol.* 2012, 683897.
- Cooper-Knock, J., Hewitt, C., Highley, J.R., Brockington, A., Milano, A., Man, S., et al., 2012. Clinico-pathological features in amyotrophic lateral sclerosis with expansions in C9orf72. *Brain* 135, 751–764.
- Cox, P., Richer, R., Metcalf, J., Banack, S., Codd, G., Bradley, W., 2009. Cyanobacteria and BMAA exposure from desert dust: a possible link to sporadic ALS among Gulf War veterans. *Amyotroph. Lateral Scler.* 10, 109–117.
- Cronin, S., Hardiman, O., Traynor, B.J., 2007. Ethnic variation in the incidence of ALS: a systematic review. *Neurology* 68, 1002–1007.
- Cruz-Aguado, R., Winkler, D., Shaw, C.A., 2006. Lack of behavioral and neuropathological effects of dietary beta-methylamino-L-alanine (BMAA) in mice. *Pharmacol. Biochem. Behav.* 84, 294–299.
- Czapinski, A., Yen, A.A., Simpson, E.P., Appel, S.H., 2006. Slower disease progression and prolonged survival in contemporary patients with amyotrophic lateral sclerosis: is the natural history of amyotrophic lateral sclerosis changing. *Arch. Neurol.* 63, 1139–1143.
- Davignul, M.L., Plassman, B.L., Pizada, A., Bell, C.C., Bowen, P.E., Burke, J.R., et al., 2011. Risk factors and preventive interventions for Alzheimer disease: state of the science. *Arch. Neurol.* 68, 1185–1190.
- Dedic, S.I., Stevic, Z., Dedic, V., Stojanovic, V.R., Milicev, M., Lavnica, D., 2012. Is hyperlipidemia correlated with longer survival in patients with amyotrophic lateral sclerosis. *Neurol. Res.* 34, 576–580.
- de Jong, S.W., Huisman, M.H., Sutedja, N.A., van der Kooi, A.J., de Visser, M., Schelhaas, H.J., et al., 2012. Smoking, alcohol consumption, and the risk of amyotrophic lateral sclerosis: a population-based study. *Am. J. Epidemiol.*
- de Munck, E., Munoz-Saez, E., Miguel, B.G., Solas, M.T., Ojeda, I., Martinez, A., et al., 2013. 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.* 36, 243–255.
- Desnuelle, C., Dib, M., Garrel, C., Favier, A., 2001. 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.* 2, 9–18.
- Diekstra, F.P., Van Deerlin, V.M., van Swieten, J.C., Al-Chalabi, A., Ludolph, A.C., Weishaupt, J.H., et al., 2014. C9orf72 and UNC13A are shared risk loci for amyotrophic lateral sclerosis and frontotemporal dementia: a genome-wide meta-analysis. *Ann. Neurol.* 76, 120–133.
- Doi, H., Kikuchi, H., Murai, H., Kawano, Y., Shigeto, H., Ohya, Y., et al., 2006. Motor neuron disorder simulating ALS induced by chronic inhalation of pyrethroid insecticides. *Neurology* 67, 1894–1895.
- Doi, Y., Yokoyama, T., Tango, T., Takahashi, K., Fujimoto, K., Nakano, I., 2010. Temporal trends and geographic clusters of mortality from amyotrophic lateral sclerosis in Japan, 1995–2004. *J. Neurol. Sci.* 298, 78–84.
- Dorst, J., Kuhnlein, P., Hendrich, C., Kassubek, J., Sperfeld, A.D., Ludolph, A.C., 2011. Patients with elevated triglyceride and cholesterol serum levels have a prolonged survival in amyotrophic lateral sclerosis. *J. Neurol.* 258, 613–617.
- Drory, V.E., Artmonov, I., 2007. Earlier onset and shorter survival of amyotrophic lateral sclerosis in Jewish patients of North African origin. A clue to modifying genetic factors? *J. Neurol. Sci.* 258, 39–43.
- Drory, V.E., Birnbaum, M., Korczyn, A.D., Chapman, J., 2001. Association of APOE epsilon4 allele with survival in amyotrophic lateral sclerosis. *J. Neurol. Sci.* 190, 17–20.
- Duncan, M.W., Steele, J.C., Kopin, I.J., Markey, S.P., 1990. 2-Amino-3-(methylamino)-propanoic acid (BMAA) in cycad flour: an unlikely cause of amyotrophic lateral sclerosis and parkinsonism-dementia of Guam. *Neurology* 40, 767–772.
- Dupuis, L., Corcia, P., Fergani, A., Gonzalez De Aguilar, J.L., Bonnefont-Rousselot, D., Bittar, R., et al., 2008. Dyslipidemia is a protective factor in amyotrophic lateral sclerosis. *Neurology* 70, 1004–1009.
- Edrissi, B., Taghizadeh, K., Dedon, P.C., 2013. Quantitative analysis of histone modifications: formaldehyde is a source of pathological n(6)-formyllysine that is refractory to histone deacetylases. *PLoS Genet.* 9, e1003328.
- Ermilova, I.P., Ermilov, V.B., Levy, M., Ho, E., Pereira, C., Beckman, J.S., 2005. Protection by dietary zinc in ALS mutant G93A SOD transgenic mice. *Neurosci. Lett.* 379, 42–46.
- Esik, O., Vönöczky, K., Lengyel, Z., Sáfrány, G., Trón, L., 2004. Characteristics of radiogenic lower motor neurone disease, a possible link with a preceding viral infection. *Spinal Cord Off. J. Int. Med. Soc. Paraplegia* 42 (February 01), 99–105.

- Fang, F., Ye, W., 2010. Smoking may be considered an established risk factor for sporadic als. *Neurology* 74, 1927.
- Fang, F., Bellocchio, R., Heman, M., Ye, W., 2006. Smoking, snuff dipping and the risk of amyotrophic lateral sclerosis – a prospective cohort study. *Neuroepidemiology* 27, 217–221.
- Fang, F., Quinlan, P., Ye, W., Barber, M.K., Umbach, D.M., Sandler, D.P., et al., 2009. Workplace exposures and the risk of amyotrophic lateral sclerosis. *Environ. Health Perspect.* 117, 1387–1392.
- Fang, F., Kwee, L.C., Allen, K.D., Umbach, D.M., Ye, W., Watson, M., et al., 2010. Association between blood lead and the risk of amyotrophic lateral sclerosis. *Am. J. Epidemiol.* 171, 1126–1133.
- Federico, A., Cardaioli, E., Da Pozzo, P., Formichi, P., Gallus, G.N., Radi, E., 2012. Mitochondria, oxidative stress and neurodegeneration. *J. Neurol. Sci.* 322, 254–262.
- Ferrari, R., Hernandez, D.G., Nalls, M.A., Rohrer, J.D., Ramasamy, A., Kwok, J.B., et al., 2014. Frontotemporal dementia and its subtypes: a genome-wide association study. *Lancet Neurol.* 13, 686–699.
- Field, N.C., Metcalf, J.S., Callier, T.A., Banack, S.A., Cox, P.A., Stommel, E.W., 2013. Linking beta-methylamino-L-alanine exposure to sporadic amyotrophic lateral sclerosis in Annapolis, MD. *Toxicol.* 70, 179–183.
- Figuerola-Romero, C., Hur, J., Bender, D.E., Delaney, C.E., Cataldo, M.D., Smith, A.L., et al., 2012. Identification of epigenetically altered genes in sporadic amyotrophic lateral sclerosis. *PLoS One* 7, e52672.
- Fitzgerald, K.C., O'Reilly, E.J., Fondell, E., Falcone, G.J., McCullough, M.L., Park, Y., et al., 2013. Intakes of vitamin C and carotenoids and risk of amyotrophic lateral sclerosis: pooled results from 5 cohort studies. *Ann. Neurol.* 73, 236–245.
- Fitzgerald, K.C., O'Reilly, E.J., Falcone, G.J., McCullough, M.L., Park, Y., Kolonel, L.N., et al., 2014. Dietary omega-3 polyunsaturated fatty acid intake and risk for amyotrophic lateral sclerosis. *JAMA Neurol.*
- Fogh, I., Ratti, A., Gellera, C., Lin, K., Tiloca, C., Moskvina, V., et al., 2014. A genome-wide association meta-analysis identifies a novel locus at 17q11: 2 associated with sporadic amyotrophic lateral sclerosis. *Hum. Mol. Genet.* 23, 2220–2231.
- Fondell, E., Fitzgerald, K.C., Falcone, G.J., O'Reilly, E.J., Ascherio, A., 2013. Early-onset alopecia and amyotrophic lateral sclerosis: a cohort study. *Am. J. Epidemiol.* 178, 1146–1149.
- Fondell, E., O'Reilly, E.J., Fitzgerald, K.C., Falcone, G.J., Kolonel, L.N., Park, Y., et al., 2015. Intakes of caffeine, coffee and tea and risk of amyotrophic lateral sclerosis: results from five cohort studies. *Amyotroph. Lateral Scler. Frontotemporal Degener.* 16, 366–371.
- Fonseca, R.G., Resende, L.A., Silva, M.D., Camargo, A., 1993. Chronic motor neuron disease possibly related to intoxication with organochlorine insecticides. *Acta Neurol. Scand.* 88, 56–58.
- Forbes, R.B., Colville, S., Cran, G.W., Swingle, R.J., 2004. Scottish Motor Neurone Disease Register: unexpected decline in survival from amyotrophic lateral sclerosis/motor neurone disease. *J. Neurol. Neurosurg. Psychiatry* 75, 1753–1755.
- Freedman, D.M., Wu, J., Daugherty, S.E., Kuncel, R.W., Enewold, L.R., Pfeiffer, R.M., 2014. The risk of amyotrophic lateral sclerosis after cancer in U.S. elderly adults: a population-based prospective study. *Int. J. Cancer* 135, 1745–1750.
- Galbussera, A., Tremolizzo, L., Brighina, L., Testa, D., Lovati, R., Ferrarese, C., et al., 2006. Vitamin E intake and quality of life in amyotrophic lateral sclerosis patients: a follow-up case series study. *Neurol. Sci.* 27, 190–193.
- Gallagher, J.P., Sanders, M., 1987. Trauma and amyotrophic lateral sclerosis: a report of 78 patients. *Acta Neurol. Scand.* 75, 145–150.
- Gallo, V., Bueno-De-Mesquita, H., Vermeulen, R., Andersen, P.M., Kyro, A., Linseisen, J., et al., 2009. Smoking and risk for amyotrophic lateral sclerosis: analysis of the EPIC cohort. *Ann. Neurol.* 65, 378–385.
- Gargiulo-Monachelli, G.M., Janota, F., Bettini, M., Shoesmith, C.L., Strong, M.J., Sica, R.E., 2012. Regional spread pattern predicts survival in patients with sporadic amyotrophic lateral sclerosis. *Eur. J. Neurol.* 19, 834–841.
- Georgouloupoulou, E., Vinceti, M., Bonvicini, F., Sola, P., Goldoni, C.A., De Girolamo, G., et al., 2011. Changing incidence and subtypes of ALS in Modena, Italy: a 10-years prospective study. *Amyotroph. Lateral Scler.* 12, 451–457.
- Gibson, S.B., Abbott, D., Farnham, J.M., Thai, K.K., McLean, H., Figuerola, K.P., et al., 2016. Population-based risks for cancer in patients with ALS. *Neurology* 87, 289–294.
- Gil, J., Vazquez, M.C., Ketzoian, C., Perna, A., Marin, B., Preux, P.M., et al., 2009. Prognosis of ALS: comparing data from the Limousin referral centre, France, and a Uruguayan population. *Amyotroph. Lateral Scler.* 10, 355–360.
- Glover, W.B., Mash, D.C., Murch, S.J., 2014. The natural non-protein amino acid N-beta-methylamino-L-alanine (BMAA) is incorporated into protein during synthesis. *Amino Acids* 46, 2553–2559.
- Goh, K.J., Tian, S., Shahrizaila, N., Ng, C.W., Tan, C.T., 2011. Survival and prognostic factors of motor neuron disease in a multi-ethnic Asian population. *Amyotroph. Lateral Scler.* 12, 124–129.
- Gordon, P.H., Artaud, F., Aouba, A., Laurent, F., Meininger, V., Elbaz, A., 2011a. Changing mortality for motor neuron disease in France (1968–2007): an age-period-cohort analysis. *Eur. J. Epidemiol.* 26, 729–737.
- Gordon, P.H., Delgadillo, D., Piquard, A., Bruneteau, G., Pradat, P.F., Salachas, F., et al., 2011b. The range and clinical impact of cognitive impairment in French patients with ALS: a cross-sectional study of neuropsychological test performance. *Amyotroph. Lateral Scler.* 12, 372–378.
- Gordon, P.H., Salachas, F., Bruneteau, G., Pradat, P.F., Lacomblez, L., Gonzalez-Bermejo, J., et al., 2012. Improving survival in a large French ALS center cohort. *J. Neurol.* 259, 1788–1792.
- Goris, A., van Setten, J., Diekstra, F., Ripke, S., Patsopoulos, N.A., Sawcer, S.J., et al., 2014. No evidence for shared genetic basis of common variants in multiple sclerosis and amyotrophic lateral sclerosis. *Hum. Mol. Genet.* 23, 1916–1922.
- Govone, F., Vacca, A., Rubino, E., Gai, A., Boschi, S., Gentile, S., et al., 2014. Lack of association between APOE gene polymorphisms and amyotrophic lateral sclerosis: a comprehensive meta-analysis. *Amyotroph. Lateral Scler. Frontotemporal Degener.* 15, 551–556.
- Grad, L.L., Guest, W.C., Yanai, A., Pokrishevsky, E., O'Neill, M.A., Gibbs, E., et al., 2011. Intermolecular transmission of superoxide dismutase 1 misfolding in living cells. *Proc. Natl. Acad. Sci. U. S. A.* 108, 16398–16403.
- Graf, M., Ecker, D., Horowski, R., Kramer, B., Riederer, P., Gerlach, M., et al., 2005. 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.* 112, 649–660.
- Gresham, L.S., Molgaard, C.A., Golbeck, A.L., Smith, R., 1986. Amyotrophic lateral sclerosis and occupational heavy metal exposure: a case-control study. *Neuroepidemiology* 5, 29–38.
- Gresham, L.S., Molgaard, C.A., Golbeck, A.L., Smith, R., 1992. Lead exposure and ALS. *Neurology* 42, 2228–2229.
- Groeneveld, G.J., de Leeuw van Weenen, J., van Muiswinkel, F.L., Veldman, H., Veldink, J.H., Wokke, J.H., et al., 2003. Zinc amplifies mSOD1-mediated toxicity in a transgenic mouse model of amyotrophic lateral sclerosis. *Neurosci. Lett.* 352, 175–178.
- Guest, W.C., Plotkin, S.S., Cashman, N.R., 2011. Toward a mechanism of prion misfolding and structural models of PrP(Sc): current knowledge and future directions. *J. Toxicol. Environ. Health A* 74, 154–160.
- Gunnarsson, L.G., Lindberg, G., Soderfeldt, B., Axelsson, O., 1991. Amyotrophic lateral sclerosis in Sweden in relation to occupation. *Acta Neurol. Scand.* 83, 394–398.
- Gunnarsson, L.G., Lygner, P.E., Veiga-Cabo, J., de Pedro-Cuesta, J., 1996. An epidemic-like cluster of motor neuron disease in a Swedish county during the period 1973–1984. *Neuroepidemiology* 15, 142–152.
- Haley, R.W., 2003. Excess incidence of ALS in young Gulf War veterans. *Neurology* 61, 750–756.
- Hall, E.D., Andrus, P.K., Oostveen, J.A., Fleck, T.J., Gurney, M.E., 1998. Relationship of oxygen radical-induced lipid peroxidative damage to disease onset and progression in a transgenic model of familial ALS. *J. Neurosci. Res.* 53, 66–77.
- Hamidou, B., Couratier, P., Besancon, C., Nicol, M., Preux, P.M., Marin, B., 2014. Epidemiological evidence that physical activity is not a risk factor for ALS. *Eur. J. Epidemiol.* 29, 459–475.
- Harwood, C.A., McDermott, C.J., Shaw, P.J., 2009. Physical activity as an exogenous risk factor in motor neuron disease (MND): a review of the evidence. *Amyotroph. Lateral Scler.* 10, 191–204.
- He, J., Mangelsdorf, M., Fan, D., Bartlett, P., Brown, M.A., 2015. Amyotrophic lateral sclerosis genetic studies: from genome-wide association mapping to genome sequencing. *Neuroscientist* 21, 599–615.
- Hersi, M., Quach, P., Wang, M.D., Gomes, J., Gaskin, J., Krewski, D., 2016. Systematic reviews of factors associated with the onset and progression of neurological conditions in humans: a methodological overview. *Neurotoxicology* (July 1) doi:<http://dx.doi.org/10.1016/j.neuro.2016.06.017> pii: S0161-813X(16)30118-8.
- Higgins, J.P., Thompson, S.G., 2002. Quantifying heterogeneity in a meta-analysis. *Stat. Med.* 21, 1539–1558.
- Horner, R.D., Kamins, K.G., Feussner, J.R., Grambow, S.C., Hoff-Lindquist, J., Harati, Y., et al., 2003. Occurrence of amyotrophic lateral sclerosis among Gulf War veterans. *Neurology* 61, 742–749.
- Horner, R.D., Grambow, S.C., Coffman, C.J., Lindquist, J.H., Oddone, E.Z., Allen, K.D., et al., 2008. Amyotrophic lateral sclerosis among 1991 Gulf War veterans: evidence for a time-limited outbreak. *Neuroepidemiology* 31, 28–32.
- Huang, R., Guo, X., Chen, X., Zheng, Z., Wei, Q., Cao, B., et al., 2015. The serum lipid profiles of amyotrophic lateral sclerosis patients: a study from south-west China and a meta-analysis. *Amyotroph. Lateral Scler. Frontotemporal Degener.* 16, 359–365.
- Huss, A., Spoerri, A., Egger, M., Roosli, M., 2009. Swiss National Cohort Study: residence near power lines and mortality from neurodegenerative diseases: longitudinal study of the Swiss population. *Am. J. Epidemiol.* 169, 167–175.
- Ingram, C.J., Weale, M.E., Plaster, C.A., Morrison, K.E., Goodall, E.F., Pall, H.S., et al., 2012. Analysis of European case-control studies suggests that common inherited variation in mitochondrial DNA is not involved in susceptibility to amyotrophic lateral sclerosis. *Amyotroph. Lateral Scler.* 13, 341–346.
- Iwami, O., Watanabe, T., Moon, C., Nakatsuka, H., Ikeda, M., 1994. 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.* 149, 121.
- Jawaid, A., Poon, M., Strutt, A.M., Rice, L.K., McDowell, E.J., Salamone, A.R., et al., 2011. Does apolipoprotein E genotype modify the clinical expression of ALS. *Eur. J. Neurol.* 18, 618–624.
- Johnson, F., Atchison, W., 2009. The role of environmental mercury, lead and pesticide exposure in development of amyotrophic lateral sclerosis. *Neurotoxicology* 30, 761–765.
- Jokanovic, M., Kusanovic, M., 2010. Neurotoxic effects in patients poisoned with organophosphorus pesticides. *Environ. Toxicol. Pharmacol.* 29, 195–201.
- Jubelt, B., Lipton, H.L., 2004. ALS: persistent scientists do not find persisting enteroviruses. *Neurology* 62, 1250–1251.
- Jubelt, B., 1992. Motor neuron diseases and viruses: poliovirus, retroviruses, and lymphomas. *Curr. Opin. Neurol. Neurosurg.* 5, 655–658.

- Kaal, E.C., Veldman, H., Soodaar, P., Joosten, E.A., Dop Bar, P.R., 1998. Oxidant treatment causes a dose-dependent phenotype of apoptosis in cultured motoneurons. *J. Neurosci. Res.* 54, 778–786.
- Kamel, F., Umbach, D.M., Munsat, T.L., Shefner, J.M., Sandler, D.P., 1999. Association of cigarette smoking with amyotrophic lateral sclerosis. *Neuroepidemiology* 18, 194–202.
- Kamel, F., Umbach, D., Munsat, T., Shefner, J., Hu, H., Sandler, D., 2002. Lead exposure and amyotrophic lateral sclerosis. *Epidemiology* 13, 311–319.
- Kamel, F., Umbach, D.M., Hu, H., Munsat, T.L., Shefner, J.M., Taylor, J.A., et al., 2005. Lead exposure as a risk factor for amyotrophic lateral sclerosis. *Neurodegener. Dis.* 2, 195–201.
- Kamel, F., Umbach, D.M., Stallone, L., Richards, M., Hu, H., Sandler, D.P., 2008. Association of lead exposure with survival in amyotrophic lateral sclerosis. *Environ. Health Perspect.* 116, 943–947.
- Kamel, F., Umbach, D.M., Bedlack, R.S., Richards, M., Watson, M., Alavanja, M.C., et al., 2012. Pesticide exposure and amyotrophic lateral sclerosis. *Neurotoxicology* 33, 457–462.
- Kaneb, H.M., Dion, P.A., Rouleau, G.A., 2012. The FUS about arginine methylation in ALS and FTL. *EMBO J.* 31, 4249–4251.
- Kang, H., Cha, E.S., Choi, G.J., Lee, W.J., 2014. Amyotrophic lateral sclerosis and agricultural environments: a systematic review. *J. Korean Med. Sci.* 29, 1610–1617.
- Keller, M.F., Ferrucci, L., Singleton, A.B., Tienari, P.J., Laaksovirta, H., Restagno, G., et al., 2014. Genome-wide analysis of the heritability of amyotrophic lateral sclerosis. *JAMA Neurol.* 71, 1123–1134.
- Kihira, T., Kanno, S., Miwa, H., Okamoto, K., Kondo, T., 2007. The role of exogenous risk factors in amyotrophic lateral sclerosis in Wakayama, Japan. *Amyotroph. Lateral Scler.* 8, 150–156.
- Kihira, T., Yoshida, S., Okamoto, K., Kazimoto, Y., Ookawa, M., Hama, K., et al., 2008. Survival rate of patients with amyotrophic lateral sclerosis in Wakayama Prefecture, Japan, 1966–2005. *J. Neurol. Sci.* 268, 95–101.
- Kim, W.K., Liu, X., Sandner, J., Pasmantier, M., Andrews, J., Rowland, L.P., et al., 2009. Study of 962 patients indicates progressive muscular atrophy is a form of ALS. *Neurology* 73, 1686–1692.
- Kim, S.M., Kim, H., Kim, J.E., Park, K.S., Sung, J.J., Kim, S.H., et al., 2011. Amyotrophic lateral sclerosis is associated with hypolipidemia at the presymptomatic stage in mice. *PLoS One* 6, e17985.
- Korczyn, A.D., Drory, V.E., 2010. Smoking and amyotrophic lateral sclerosis. *Ann. Neurol.* 67, 694.
- Korner, S., Kollwe, K., Ilse, J., Muller-Heine, A., Dengler, R., Krampfl, K., et al., 2013. Prevalence and prognostic impact of comorbidities in amyotrophic lateral sclerosis. *Eur. J. Neurol.* 20, 647–654.
- Kurlander, H.M., Patten, B.M., 1979. Metals in spinal cord tissue of patients dying of motor neuron disease. *Ann. Neurol.* 6, 21–24.
- Lacomblez, L., Doppler, V., Beuclet, I., Costes, G., Salachas, F., Raisonnier, A., et al., 2002. APOE: a potential marker of disease progression in ALS. *Neurology* 58, 1112–1114.
- Laffita-Mesa, J.M., Rodriguez Pupo, J.M., Moreno Sera, R., Vazquez Mojena, Y., Kouri, V., Laguna-Salvia, L., et al., 2013. De novo mutations in ataxin-2 gene and ALS risk. *PLoS One* 8, e70560.
- Lambrechts, D., Poesen, K., Fernandez-Santiago, R., Al-Chalabi, A., Del Bo, R., Van Vught, P., et al., 2009. Meta-analysis of vascular endothelial growth factor variations in amyotrophic lateral sclerosis: increased susceptibility in male carriers of the-2578AA genotype. *J. Med. Genet.* 46, 840–846.
- Lee, Y.H., Kim, J.H., Seo, Y.H., Choi, S.J., Ji, J.D., 2015. Song GG. Paraoxonase 1 Q192R and L55M polymorphisms and susceptibility to amyotrophic lateral sclerosis: a meta-analysis. *Neurol. Sci.* 36, 11–20.
- Li, C.Y., Sung, F.C., 2003. Association between occupational exposure to power frequency electromagnetic fields and amyotrophic lateral sclerosis: a review. *Am. J. Ind. Med.* 43, 212–220.
- Li, M., Wang, L., Wang, W., Qi, X.L., Tang, Z.Y., 2014. Mutations in the HFE gene and sporadic amyotrophic lateral sclerosis risk: a meta-analysis of observational studies. *Braz. J. Med. Biol. Res.* 47, 215–222.
- Lill, C.M., Abel, O., Bertram, L., Al-Chalabi, A., 2011. Keeping up with genetic discoveries in amyotrophic lateral sclerosis: the ALSod and ALSGene databases. *Amyotroph. Lateral Scler.* 12, 238–249.
- Lill, C.M., Rengmark, A., Pihlstrom, L., Fogh, I., Shatunov, A., Sleiman, P.M., et al., 2015. The role of TREM2 R47H as a risk factor for Alzheimer's disease, frontotemporal lobar degeneration, amyotrophic lateral sclerosis, and Parkinson's disease. *Alzheimers Dement.* 11, 1407–1416.
- Livesley, B., Sissons, C.E., 1968. Chronic lead intoxication mimicking motor neurone disease. *Br. Med. J.* 4, 387–388.
- Logrosino, G., Traynor, B.J., Hardiman, O., Chio, A., Couratier, P., Mitchell, J.D., et al., 2008. Descriptive epidemiology of amyotrophic lateral sclerosis: new evidence and unsolved issues. *J. Neurol. Neurosurg. Psychiatry* 79, 6–11.
- Longstreth, W.T., McGuire, V., Koepsell, T.D., Wang, Y., van Belle, G., 1998. Risk of amyotrophic lateral sclerosis and history of physical activity: a population-based case-control study. *Arch. Neurol.* 55, 201–206.
- Ludolph, A.C., Bretschneider, J., Weishaupt, J.H., 2012. Amyotrophic lateral sclerosis. *Curr. Opin. Neurol.* 25, 530–535.
- Mackenzie, I.R., Ansoorge, O., Strong, M., Bilbao, J., Zinman, L., Ang, L.C., et al., 2011. Pathological heterogeneity in amyotrophic lateral sclerosis with FUS mutations: two distinct patterns correlating with disease severity and mutation. *Acta Neuropathol.* 122, 87–98.
- Maiti, T.K., Konar, S., Bir, S., Kalakoti, P., Bollam, P., Nanda, A., 2015. Role of apolipoprotein E polymorphism as a prognostic marker in traumatic brain injury and neurodegenerative disease: a critical review. *Neurosurg. Focus* 39, E3.
- Malaspina, A., Alimonti, D., Poloni, T., Ceroni, M., 2002. Disease clustering: the example of ALS, PD, dementia and hereditary ataxias in Italy. *Funct. Neurol.* 17, 177–182.
- Malek, A.M., Barchowsky, A., Bowser, R., Youk, A., Talbott, E.O., 2012. 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.*
- Malek, A.M., Barchowsky, A., Bowser, R., Heiman-Patterson, T., Lacomis, D., Rana, S., et al., 2015. Exposure to hazardous air pollutants and the risk of amyotrophic lateral sclerosis. *Environ. Pollut.* 197, 181–186.
- Marin, B., Couratier, P., Preux, P.M., Logrosino, G., 2011a. Can mortality data be used to estimate amyotrophic lateral sclerosis incidence. *Neuroepidemiology* 36, 29–38.
- Marin, B., Desport, J.C., Kajeu, P., Jesus, P., Nicolaud, B., Nicol, M., et al., 2011b. Alteration of nutritional status at diagnosis is a prognostic factor for survival of amyotrophic lateral sclerosis patients. *J. Neurol. Neurosurg. Psychiatry* 82, 628–634.
- Masseret, E., Banack, S., Boumediene, F., Abadie, E., Briant, L., Pernet, F., et al., 2013. Dietary BMAA exposure in an amyotrophic lateral sclerosis cluster from southern France. *PLoS One* 8, e83406.
- McCombe, P.A., Henderson, R.D., 2010. Effects of gender in amyotrophic lateral sclerosis. *Gend. Med.* 7, 557–570.
- McGuire, V., Longstreth Jr., W.T., Nelson, L.M., Koepsell, T.D., Checkoway, H., Morgan, M.S., et al., 1997. Occupational exposures and amyotrophic lateral sclerosis: a population-based case-control study. *Am. J. Epidemiol.* 145, 1076–1088.
- McKee, A.C., Cantu, R.C., Nowinski, C.J., Hedley-Whyte, E.T., Gavett, B.E., Budson, A.E., et al., 2009. Chronic traumatic encephalopathy in athletes: progressive tauopathy after repetitive head injury. *J. Neuropathol. Exp. Neurol.* 68, 709–735.
- McKee, A.C., Stein, T.D., Nowinski, C.J., Stern, R.A., Daneshvar, D.H., Alvarez, V.E., et al., 2013. The spectrum of disease in chronic traumatic encephalopathy. *Brain* 136, 43–64.
- Michal Freedman, D., Kuncel, R.W., Weinstein, S.J., Malila, N., Virtamo, J., Albanes, D., 2013. Vitamin E serum levels and controlled supplementation and risk of amyotrophic lateral sclerosis. *Amyotroph. Lateral Scler. Frontotemporal Degener.*
- Miranda, M.L., Alicia Overstreet Galeano, M., Tassone, E., Allen, K.D., Horner, R.D., 2008. Spatial analysis of the etiology of amyotrophic lateral sclerosis among 1991 Gulf War veterans. *Neurotoxicology* 29, 964–970.
- Mitchell, J.D., East, B.W., Harris, I.A., Pentland, B., 1991. Manganese, selenium and other trace elements in spinal cord, liver and bone in motor neurone disease. *Eur. Neurol.* 31, 7–11.
- Mitchell, J.D., Callaghan, P., Gardham, J., Mitchell, C., Dixon, M., Addison-Jones, R., et al., 2010. Timelines in the diagnostic evaluation of people with suspected amyotrophic lateral sclerosis (ALS)/motor neuron disease (MND)—a 20-year review: can we do better? *Amyotroph. Lateral Scler.* 11, 537–541.
- Mitchell, J.D., 1987. Heavy metals and trace elements in amyotrophic lateral sclerosis. *Neurol. Clin.* 5, 43–60.
- Morahan, J.M., Pamphlett, R., 2006. Amyotrophic lateral sclerosis and exposure to environmental toxins: an Australian case-control study. *Neuroepidemiology* 27, 130–135.
- Morahan, J.M., Yu, B., Trent, R.J., Pamphlett, R., 2007. A gene-environment study of the paraoxonase 1 gene and pesticides in amyotrophic lateral sclerosis. *Neurotoxicology* 28, 532–540.
- Morozova, N., Weisskopf, M., McCullough, M., Munger, K., Calle, E., Thun, M., et al., 2008. Diet and amyotrophic lateral sclerosis. *Epidemiology* 19, 324–337.
- Moulard, B., Sefiani, A., Laamri, A., Malafosse, A., Camu, W., 1996. Apolipoprotein E genotyping in sporadic amyotrophic lateral sclerosis: evidence for a major influence on the clinical presentation and prognosis. *J. Neurol. Sci.* 139 (Suppl), 34–37.
- Murch, S.J., Cox, P.A., Banack, S.A., Steele, J.C., Sacks, O.W., 2004. Occurrence of beta-methylamino-L-alanine (BMAA) in ALS/PDC patients from Guam. *Acta Neurol. Scand.* 110, 267–269.
- Murray, M.E., DeJesus-Hernandez, M., Rutherford, N.J., Baker, M., Duara, R., Graff-Radford, N.R., et al., 2011. Clinical and neuropathologic heterogeneity of c9FTD/ALS associated with hexanucleotide repeat expansion in C9ORF72. *Acta Neuropathol.* 122, 673–690.
- Nalini, A., Thennarasu, K., Gourie-Devi, M., Shenoy, S., Kulshreshtha, D., 2008. Clinical characteristics and survival pattern of 1,153 patients with amyotrophic lateral sclerosis: experience over 30 years from India. *J. Neurol. Sci.* 272, 60–70.
- Nelson, L., Matkin, C., Longstreth, W., McGuire, V., 2000. Population-based case-control study of amyotrophic lateral sclerosis in western Washington State. *II. Diet. Am. J. Epidemiol.* 151, 164–173.
- Neuenschwander, A.G., Thai, K.K., Figueroa, K.P., Pulst, S.M., 2014. Amyotrophic lateral sclerosis risk for spinocerebellar ataxia type 2 ATXN2 CAG repeat alleles: a meta-analysis. *JAMA Neurol.* 71, 1529–1534.
- Nix, W.A., Berger, M.M., Oberste, M.S., Brooks, B.R., McKenna-Yasek, D.M., Brown Jr., R.H., et al., 2004. Failure to detect enterovirus in the spinal cord of ALS patients using a sensitive RT-PCR method. *Neurology* 62, 1372–1377.
- Nonaka, T., Masuda-Suzukake, M., Arai, T., Hasegawa, Y., Akatsu, H., Obi, T., et al., 2013. Prion-like properties of pathological TDP-43 aggregates from diseased brains. *Cell Rep.* 4, 124–134.
- Noonan, C.W., White, M.C., Thurman, D., Wong, L.Y., 2005. Temporal and geographic variation in United States motor neuron disease mortality, 1969–1998. *Neurology* 64, 1215–1221.

- O'Reilly, E.J., Wang, H., Weisskopf, M.G., Fitzgerald, K.C., Falcone, G., McCullough, M. L., et al., 2013. Premorbid body mass index and risk of amyotrophic lateral sclerosis. *Amyotroph. Lateral Scler. Frontotemporal Degener.* 14, 205–211.
- Oh, S.S., Kim, E.A., Lee, S.W., Kim, M.K., Kang, S.K., 2007. A case of amyotrophic lateral sclerosis in electronic parts manufacturing worker exposed to lead. *Neurotoxicology* 28, 324–327.
- Oh, S.I., Baek, S., Park, J.S., Piao, L., Oh, K.W., Kim, S.H., 2015. Prognostic role of serum levels of uric acid in amyotrophic lateral sclerosis. *J. Clin. Neurol.* 11, 376–382.
- Okamoto, K., Kihira, T., Kobashi, G., Washio, M., Sasaki, S., Yokoyama, T., et al., 2009a. Fruit and vegetable intake and risk of amyotrophic lateral sclerosis in Japan. *Neuroepidemiology* 32, 251–256.
- Okamoto, K., Kihira, T., Kondo, T., Kobashi, G., Washio, M., Sasaki, S., et al., 2009b. Lifestyle factors and risk of amyotrophic lateral sclerosis: a case-control study in Japan. *Ann. Epidemiol.* 19, 359–364.
- Oteiza, P.I., Uchitel, O.D., Carrasquedo, F., Dubrovski, A.L., Roma, J.C., Fraga, C.G., 1997. Evaluation of antioxidants, protein, and lipid oxidation products in blood from sporadic amyotrophic lateral sclerosis patients. *Neurochem. Res.* 22, 535–539.
- Paganoni, S., Deng, J., Jaffa, M., Cudkowicz, M.E., Wills, A.M., 2011. Body mass index, not dyslipidemia, is an independent predictor of survival in amyotrophic lateral sclerosis. *Muscle Nerve* 44, 20–24.
- Paganoni, S., Deng, J., Jaffa, M., Cudkowicz, M.E., Wills, A.M., 2012. What does body mass index measure in amyotrophic lateral sclerosis and why should we care? *Muscle Nerve* 45, 612.
- Pall, H.S., Williams, A.C., Waring, R., Elias, E., 1987. Motoneuron disease as manifestation of pesticide toxicity. *Lancet* 2, 685.
- Pamphlett, R., Png, F.Y., 1998. Shrinkage of motor axons following systemic exposure to inorganic mercury. *J. Neuropathol. Exp. Neurol.* 57, 360–366.
- Pan, L., Deng, X., Ding, D., Leng, H., Zhu, X., Wang, Z., 2015. Association between the Angiogenin (ANG) K171 variant and amyotrophic lateral sclerosis risk in Caucasian: a meta-analysis. *Neurol. Sci.* 36, 2163–2168.
- Pan, L.S., Deng, X.B., Wang, Z., Leng, H.L., Zhu, X.P., Ding, D., 2016. Lack of association between the Angiogenin (ANG) rs11701 polymorphism and amyotrophic lateral sclerosis risk: a meta-analysis. *Neurol. Sci.* 37, 655–662.
- Park, R., Schulte, P., Bowman, J., Walker, J., Bondy, S., Yost, M., et al., 2005. Potential occupational risks for neurodegenerative diseases. *Am. J. Ind. Med.* 48, 63–77.
- Parker, L., McGuckin, T.A., Leicht, A.S., 2014. Influence of exercise intensity on systemic oxidative stress and antioxidant capacity. *Clin. Physiol. Funct. Imaging* 34, 377–383.
- Pastula, D.M., Coffman, C.J., Allen, K.D., Oddone, E.Z., Kasarskis, E.J., Lindquist, J.H., et al., 2009. Factors associated with survival in the National Registry of Veterans with ALS. *Amyotroph. Lateral Scler.* 10, 332–338.
- Perry, T.L., Bergeron, C., Biro, A.J., Hansen, S., 1989. Beta-N-methylamino-L-alanine: chronic oral administration is not neurotoxic to mice. *J. Neurol. Sci.* 94, 173–180.
- Pfister, T., Sekhon, R., White, M., Scott, P., Munro, S., Johnston, M., et al., 2013. Familial amyotrophic lateral sclerosis in Alberta, Canada. *Amyotroph. Lateral Scler. Frontotemporal Degener.* 14, 273–277.
- Phukan, J., Pender, N.P., Hardiman, O., 2007. Cognitive impairment in amyotrophic lateral sclerosis. *Lancet Neurol.* 6, 994–1003.
- Phukan, J., Elamin, M., Bede, P., Jordan, N., Gallagher, L., Byrne, S., et al., 2011. The syndrome of cognitive impairment in amyotrophic lateral sclerosis: a population-based study. *J. Neurol. Neurosurg. Psychiatry*.
- Piaceri, I., Del Mastio, M., Tedde, A., Bagnoli, S., Latorraca, S., Massaro, F., et al., 2011. Clinical heterogeneity in Italian patients with amyotrophic lateral sclerosis. *Clin. Genet.*
- Pierce-Ruhland, R., Patten, B.M., 1981. Repeat study of antecedent events in motor neuron disease. *Ann. Clin. Res.* 13, 102–107.
- Pinkerton, L.E., Hein, M.J., Meyers, A., Kamel, F., 2013. Assessment of ALS mortality in a cohort of formaldehyde-exposed garment workers. *Amyotroph. Lateral Scler. Frontotemporal Degener.* 14, 353–355.
- Plato, C.C., Garruto, R.M., Galasko, D., Craig, U., Plato, M., Gamst, A., et al., 2003. Amyotrophic lateral sclerosis and parkinsonism-dementia complex of Guam: changing incidence rates during the past 60 years. *Am. J. Epidemiol.* 157, 149–157.
- Poloni, M., Micheli, A., Facchetti, D., Mai, R., Ceriani, F., Cattalini, C., 1997. Conjugal amyotrophic lateral sclerosis: toxic clustering or change. *Ital. J. Neurol. Sci.* 18, 109–112.
- Pouilletier de Gannes, F., Ruffie, G., Taxile, M., Ladeveze, E., Hurtier, A., Haro, E., et al., 2009. Amyotrophic lateral sclerosis (ALS) and extremely-low frequency (ELF) magnetic fields: a study in the SOD-1 transgenic mouse model. *Amyotroph. Lateral Scler.* 10, 370–373.
- Praline, J., Guennoc, A.M., Limousin, N., Hallak, H., de Toffol, B., Corcia, P., 2007. ALS and mercury intoxication: a relationship. *Clin. Neurol. Neurosurg.* 109, 880–883.
- Praline, J., Blasco, H., Vourc'h, P., Garrigue, M.A., Gordon, P.H., Camu, W., et al., 2011. APOE epsilon4 allele is associated with an increased risk of bulbar-onset amyotrophic lateral sclerosis in men. *Eur. J. Neurol.* 18, 1046–1052.
- Provinciale, L., Giovagnoli, A.R., 1990. Antecedent events in amyotrophic lateral sclerosis: do they influence clinical onset and progression. *Neuroepidemiology* 9, 255–262.
- Pupillo, E., Messina, P., Logroscino, G., Zoccollella, S., Chio, A., Calvo, A., et al., 2012. Trauma and amyotrophic lateral sclerosis: a case-control study from a population-based registry. *Eur. J. Neurol.* 19, 1509–1517.
- Qureshi, M.M., Hayden, D., Urbinelli, L., Ferrante, K., Newhall, K., Myers, D., et al., 2006. Analysis of factors that modify susceptibility and rate of progression in amyotrophic lateral sclerosis (ALS). *Amyotroph. Lateral Scler.* 7, 173–182.
- Ratti, A., Corrado, L., Castellotti, B., Del Bo, R., Fogh, I., Cereda, C., et al., 2012. C9ORF72 repeat expansion in a large Italian ALS cohort: evidence of a founder effect. *Neurobiol. Aging* 33, 2528. e7–14.
- Reisner, H.M., Lundblad, R.L., 2009. Identifying residues in antigenic determinants by chemical modification. *Methods Mol. Biol.* 524, 103–117.
- Rentzos, M., Evangelopoulos, E., Sereti, E., Zouvelou, V., Marmara, S., Alexakis, T., et al., 2011. Alterations of T cell subsets in ALS: a systemic immune activation? *Acta Neurol. Scand.*
- Ricci, C., Battistini, S., Cozzi, L., Benigni, M., Origone, P., Verriello, L., et al., 2011. Lack of association of PON polymorphisms with sporadic ALS in an Italian population. *Neurobiol. Aging* 32, 552 (e7552.13).
- Robberecht, W., 2000. Genetics of amyotrophic lateral sclerosis. *J. Neurol.* 247 (Suppl 6), VI/2–VI/6.
- Roberts, A.L., Johnson, N.J., Cudkowicz, M.E., Eum, K.D., Weisskopf, M.G., 2015. Job-related formaldehyde exposure and ALS mortality in the USA. *J. Neurol. Neurosurg. Psychiatry*.
- Rose, M.R., Brix, K.A., 2006. Neurological disorders in gulf war veterans. *Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci.* 361 (April), 29.
- Rose, K.A., 1994. Electrical shock injury. *J. Manipulative Physiol. Ther.* 17, 174–176.
- Sabatelli, M., Madia, F., Conte, A., Luigetti, M., Zollino, M., Mancuso, L., et al., 2008. Natural history of young-adult amyotrophic lateral sclerosis. *Neurology* 71, 876–881.
- Sabatelli, M., Conte, A., Zollino, M., 2013. Clinical and genetic heterogeneity of amyotrophic lateral sclerosis. *Clin. Genet.* 83, 408–416.
- Saeed, M., Siddique, N., Hung, W.Y., Usacheva, E., Liu, E., Sufit, R.L., et al., 2006. Paraoxonase cluster polymorphisms are associated with sporadic ALS. *Neurology* 67, 771–776.
- Sathasivam, S., 2010. Motor neuron disease: clinical features, diagnosis, diagnostic pitfalls and prognostic markers. *Singapore Med. J.* 51 (367), 72 (quiz 373).
- Scarmeas, N., Shih, T., Stern, Y., Ottman, R., Rowland, L.P., 2002. Premorbid weight, body mass, and varsity athletics in ALS. *Neurology* 59, 773–775.
- Scarpa, M., Colombo, A., Panzetti, P., Sorgato, P., 1988. Epidemiology of amyotrophic lateral sclerosis in the province of Modena, Italy. Influence of environmental exposure to lead. *Acta Neurol. Scand.* 77, 456–460.
- Schmidt, S., Kwee, L.C., Allen, K.D., Oddone, E.Z., 2010. Association of ALS with head injury, cigarette smoking and APOE genotypes. *J. Neurol. Sci.* 291, 22–29.
- Seals, R.M., Hansen, J., Gredal, O., Weisskopf, M.G., 2016. Physical trauma and amyotrophic lateral sclerosis: a population-based study using danish national registries. *Am. J. Epidemiol.* 183, 294–301.
- Seltman, R.E., Matthews, B.R., 2012. Frontotemporal lobar degeneration: epidemiology, pathology, diagnosis and management. *CNS Drugs* 26, 841–870.
- Shea, B.J., Bouter, L.M., Peterson, J., Boers, M., Andersson, N., Ortiz, Z., et al., 2007. External validation of a measurement tool to assess systematic reviews (AMSTAR). *PLoS One* 2, e1350.
- Shimizu, T., Nagaoka, U., Nakayama, Y., Kawata, A., Kugimoto, C., Kuroiwa, Y., et al., 2012. Reduction rate of body mass index predicts prognosis for survival in amyotrophic lateral sclerosis: a multicenter study in Japan. *Amyotroph. Lateral Scler.* 13, 363–366.
- Silani, V., Messina, S., Poletti, B., Morelli, C., Doretti, A., Ticozzi, N., et al., 2011. The diagnosis of Amyotrophic lateral sclerosis in 2010. *Arch. Ital. Biol.* 149, 5–27.
- Sirdofsky, M.D., Hawley, R.J., Manz, H., 1991. Progressive motor neuron disease associated with electrical injury. *Muscle Nerve* 14, 977–980.
- Sleegers, K., Brouwers, N., Maurer-Stroh, S., van Es, M.A., Van Damme, P., van Vught, P.W., et al., 2008. Progranulin genetic variability contributes to amyotrophic lateral sclerosis. *Neurology* 71, 253–259.
- Smethurst, P., Sidle, K.C., Hardy, J., 2014. Prion-like mechanisms of TDP-43 in ALS. *Neuropathol. Appl. Neurobiol.*
- Smith, B.N., Newhouse, S., Shatunov, A., Vance, C., Topp, S., Johnson, L., et al., 2012. The C9ORF72 expansion mutation is a common cause of ALS+/-FTD in Europe and has a single founder. *Eur. J. Hum. Genet.*
- Smith, B.N., Vance, C., Scotter, E.L., Troakes, C., Wong, C.H., Topp, S., et al., 2015. Novel mutations support a role for Profilin 1 in the pathogenesis of ALS. *Neurobiol. Aging* 36, 1602 (e171602. e27).
- Spencer, P.S., Hugon, J., Ludolph, A., Nunn, P.B., Ross, S.M., Roy, D.N., et al., 1987a. Discovery and partial characterization of primate motor-system toxins. *Ciba Found. Symp.* 126, 221–238.
- Spencer, P.S., Nunn, P.B., Hugon, J., Ludolph, A.C., Ross, S.M., Roy, D.N., et al., 1987b. Guam amyotrophic lateral sclerosis-parkinsonism-dementia linked to a plant excitant neurotoxin. *Science* 237, 517–522.
- Spencer, P.S., Palmer, V.S., Ludolph, A.C., 2005. On the decline and etiology of high-incidence motor system disease in West Papua (southwest New Guinea). *Mov. Disord. Off. J. Mov. Disord. Soc.* 20 (Suppl 12), S119–S126 (August 01, 2005).
- Spencer, P.S., 1987. Guam ALS/parkinsonism-dementia: a long-latency neurotoxic disorder caused by slow toxin(s) in food. *Can. J. Neurol. Sci.* 14, 347–357.
- Steele, J.C., 2005. Parkinsonism-dementia complex of Guam. *Mov. Disorders: Off. J. Mov. Disord. Soc.* 20 (Suppl 12), S99–S107 (August 01, 2005).
- Stewart, H., Rutherford, N.J., Briemberg, H., Krieger, C., Cashman, N., Fabros, M., et al., 2012. Clinical and pathological features of amyotrophic lateral sclerosis caused by mutation in the C9ORF72 gene on chromosome 9p. *Acta Neuropathol.*
- Stohs, S.J., Bagchi, D., 1995. Oxidative mechanisms in the toxicity of metal ions. *Free Radic. Biol. Med.* 18, 321–336.
- Stone, R., 1993. Guam: deadly disease dying out. *Science* 261, 424–426.
- Strickland, D., Smith, S.A., Dolliff, G., Goldman, L., Roelofs, R.L., 1996. Physical activity, trauma, and ALS: a case-control study. *Acta Neurol. Scand.* 94, 45–50.
- Stroup, D.F., Berlin, J.A., Morton, S.C., Olkin, I., Williamson, G.D., Rennie, D., et al., 2000. Meta-analysis of observational studies in epidemiology: a proposal for

- reporting: meta-analysis of observational studies in epidemiology (MOOSE) group. *JAMA* 283, 2008–2012.
- Su, F.C., Goutman, S.A., Chernyak, S., Mukherjee, B., Callaghan, B.C., Batterman, S., et al., 2016. Association of environmental toxins with amyotrophic lateral sclerosis. *JAMA Neurol.* 73, 803–811.
- Sutedja, N.A., Veldink, J.H., Fischer, K., Kromhout, H., Wokke, J.H., Huisman, M.H., et al., 2007. Lifetime occupation, education, smoking, and risk of ALS. *Neurology* 69, 1508–1514.
- Sutedja, N.A., Veldink, J.H., Fischer, K., Kromhout, H., Heederik, D., Huisman, M.H., et al., 2009a. Exposure to chemicals and metals and risk of amyotrophic lateral sclerosis: a systematic review. *Amyotroph. Lateral Scler.* 10, 302–309.
- Sutedja, N.A., Fischer, K., Veldink, J.H., van der Heijden, G.J.M.G., Kromhout, H., Heederik, D., et al., 2009b. What we truly know about occupation as a risk factor for ALS: a critical and systematic review. *Amyotroph. Lateral Scler. Off. Publ. World Fed. Neurol. Res. Group Motor Neuron Dis.* 10, 295–301.
- Sutedja, N.A., van der Schouw, Y.T., Fischer, K., Sizoo, E.M., Huisman, M.H., Veldink, J. H., et al., 2011. Beneficial vascular risk profile is associated with amyotrophic lateral sclerosis. *J. Neurol. Neurosurg. Psychiatry* 82, 638–642.
- Talman, P., Forbes, A., Mathers, S., 2009. Clinical phenotypes and natural progression for motor neuron disease: analysis from an Australian database. *Amyotroph. Lateral Scler.* 10, 79–84.
- Terry, P.D., Kamel, F., Umbach, D.M., Lehman, T.A., Hu, H., Sandler, D.P., et al., 2004. VEGF promoter haplotype and amyotrophic lateral sclerosis (ALS). *J. Neurogenet.* 18, 429–434.
- Testa, D., Lovati, R., Ferrarini, M., Salmoiraghi, F., Filippini, G., 2004. Survival of 793 patients with amyotrophic lateral sclerosis diagnosed over a 28-year period. *Amyotroph. Lateral Scler. Other Motor Neuron Disord.* 5, 208–212.
- Tetsuka, S., Morita, M., Iida, A., Uehara, R., Ikegawa, S., Nakano, I., 2013. ZNF512B gene is a prognostic factor in patients with amyotrophic lateral sclerosis. *J. Neurol. Sci.* 324, 163–166.
- Ticozzi, N., LeClerc, A.L., Keagle, P.J., Glass, J.D., Wills, A.M., van Blitterswijk, M., et al., 2010. Paraonoxase gene mutations in amyotrophic lateral sclerosis. *Ann. Neurol.* 68, 102–107.
- Tosi, L., Righetti, C., Adams, L., Zanette, G., 1994. 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* 57, 810–813.
- Trumbull, K.A., Beckman, J.S., 2009. A role for copper in the toxicity of zinc-deficient superoxide dismutase to motor neurons in amyotrophic lateral sclerosis. *Antioxid. Redox Signal.* 11, 1627–1639.
- Tsong, T.Y., Su, Z.D., 1999. Biological effects of electric shock and heat denaturation and oxidation of molecules, membranes, and cellular functions. *Ann. N. Y. Acad. Sci.* 888, 211–232.
- Turabelidze, G., Zhu, B.P., Schootman, M., Malone, J.L., Horowitz, S., Weidinger, J., et al., 2008. An epidemiologic investigation of amyotrophic lateral sclerosis in Jefferson County, Missouri, 1998–2002. *Neurotoxicology* 29, 81–86.
- Turner, M.R., Abisgold, J., Yeates, D.G., Talbot, K., Goldacre, M.J., 2010. Head and other physical trauma requiring hospitalisation is not a significant risk factor in the development of ALS. *J. Neurol. Sci.* 288, 45–48.
- Turner, M.C., Wigle, D.T., Krewski, D., 2011. Residential pesticides and childhood leukemia: a systematic review and meta-analysis. *Cien. Saude Colet.* 16, 1915–1931.
- Turner, M.R., Wotton, C., Talbot, K., Goldacre, M.J., 2012. Cardiovascular fitness as a risk factor for amyotrophic lateral sclerosis: indirect evidence from record linkage study. *J. Neurol. Neurosurg. Psychiatry* 83, 395–398.
- Turner, M.R., Hardiman, O., Benatar, M., Brooks, B.R., Chio, A., de Carvalho, M., et al., 2013. Controversies and priorities in amyotrophic lateral sclerosis. *Lancet Neurol.* 12, 310–322.
- Turner, M.R., Goldacre, R., Talbot, K., Goldacre, M.J., 2015. Cerebrovascular injury as a risk factor for amyotrophic lateral sclerosis. *J. Neurol. Neurosurg. Psychiatry* .
- Uccelli, R., Binazzi, A., Altavista, P., Belli, S., Comba, P., Mastrantonio, M., et al., 2007. Geographic distribution of amyotrophic lateral sclerosis through motor neuron disease mortality data. *Eur. J. Epidemiol.* 22, 781–790.
- Valenti, M., Pontieri, F.E., Conti, F., Altobelli, E., Manzoni, T., Frati, L., 2005. Amyotrophic lateral sclerosis and sports: a case-control study. *Eur. J. Neurol.* 12, 223–225.
- Valentine, J.S., Doucette, P.A., Zittin Potter, S., 2005. Copper-zinc superoxide dismutase and amyotrophic lateral sclerosis. *Annu. Rev. Biochem.* 74 (Jan 01), 563–593.
- Vanacore, N., Binazzi, A., Bottazzi, M., Belli, S., 2006. Amyotrophic lateral sclerosis in an Italian professional soccer player. *Parkinsonism Relat. Disord.* 12, 327–329.
- Vanacore, N., Cocco, P., Fadda, D., Dosemeci, M., 2010. Job strain, hypoxia and risk of amyotrophic lateral sclerosis: results from a death certificate study. *Amyotroph. Lateral Scler.* 11, 430–434.
- Vandenbergh, N., Leveque, N., Corcia, P., Brunaud-Danel, V., Salort-Campana, E., Besson, G., et al., 2010. Cerebrospinal fluid detection of enterovirus genome in ALS: a study of 242 patients and 354 controls. *Amyotroph. Lateral Scler.* 11, 277–282.
- van Blitterswijk, M., Blokhuis, A., van Es, M.A., van Vught, P.W., Rowicka, P.A., Schelhaas, H.J., et al., 2012a. Rare and common paraonoxase gene variants in amyotrophic lateral sclerosis patients. *Neurobiol. Aging* .
- van Blitterswijk, M., DeJesus-Hernandez, M., Rademakers, R., 2012b. How do C9ORF72 repeat expansions cause amyotrophic lateral sclerosis and frontotemporal dementia: can we learn from other noncoding repeat expansion disorders. *Curr. Opin. Neurol.* 25, 689–700.
- van der Zee, J., Van Langenhove, T., Kovacs, G.G., Dillen, L., Deschamps, W., Engelborghs, S., et al., 2014. Rare mutations in SQSTM1 modify susceptibility to frontotemporal lobar degeneration. *Acta Neuropathol.* 128, 397–410.
- van Es, M.A., Veldink, J.H., Saris, C.G., Blauw, H.M., van Vught, P.W., Birve, A., et al., 2009. Genome-wide association study identifies 19p13.3 (UNC13A) and 9p21.2 as susceptibility loci for sporadic amyotrophic lateral sclerosis. *Nat. Genet.* 41, 1083–1087.
- van Rhee, W., Diekstra, F.P., van Doormaal, P.T., Seelen, M., Kenna, K., McLaughlin, R., et al., 2013. H63D polymorphism in HFE is not associated with amyotrophic lateral sclerosis. *Neurobiol. Aging* 34, 1517 (e5,1517. e7).
- Veldink, J.H., Kalmijn, S., Van der Hout, A.H., Lemmink, H.H., Groeneveld, G.J., Lummens, C., et al., 2005a. SMN genotypes producing less SMN protein increase susceptibility to and severity of sporadic ALS. *Neurology* 65, 820–825.
- Veldink, J., Kalmijn, S., Groeneveld, G., Titulaer, M., Wokke, J., van den Berg, L., 2005b. Physical activity and the association with sporadic ALS. *Neurology* 64, 241–245.
- Veldink, J.H., Kalmijn, S., Groeneveld, G., Wunderink, W., Koster, A., de Vries, J., et al., 2007. Intake of polyunsaturated fatty acids and vitamin E reduces the risk of developing amyotrophic lateral sclerosis. *J. Neurol. Neurosurg. Psychiatry* 78, 367–371.
- Vergara, X., Kheifets, L., Greenland, S., Oksuzyan, S., Cho, Y.S., Mezei, G., 2013. Occupational exposure to extremely low-frequency magnetic fields and neurodegenerative disease: a meta-analysis. *J. Occup. Environ. Med.* 55, 135–146.
- Vinceti, M., Bonvicini, F., Bergomi, M., Malagoli, C., 2010. Possible involvement of overexposure to environmental selenium in the etiology of amyotrophic lateral sclerosis: a short review. *Ann. Ist. Super. Sanita* 46, 279–283.
- Vinceti, M., Bottecchi, I., Fan, A., Finkelstein, Y., Mandrioli, J., 2012. Are environmental exposures to selenium, heavy metals, and pesticides risk factors for amyotrophic lateral sclerosis. *Rev. Environ. Health* 27, 19–41.
- Wang, M.D., Little, J., 2016. How credible are meta-Analyses of risk factors based on observational studies for amyotrophic lateral sclerosis a new insight from an umbrella review. *Neuroepidemiology* 46, 271–272.
- Wang, Y., Mao, X.O., Xie, L., Banwait, S., Marti, H.H., Greenberg, D.A., et al., 2007. Vascular endothelial growth factor overexpression delays neurodegeneration and prolongs survival in amyotrophic lateral sclerosis mice. *J. Neurosci.* 27, 304–307.
- Wang, H., Weisskopf, M., O'Reilly, E., Logroschino, G., McCullough, M., Schatzkin, A., et al., 2008. Prospective studies on smoking and risk off amyotrophic lateral sclerosis. *Neurology* 70, A190.
- Wang, H., O'Reilly, E.J., Weisskopf, M.G., Logroschino, G., McCullough, M.L., Schatzkin, A., et al., 2011a. Vitamin E intake and risk of amyotrophic lateral sclerosis: a pooled analysis of data from 5 prospective cohort studies. *Am. J. Epidemiol.* 173, 595–602.
- Wang, H., O'Reilly, E.J., Weisskopf, M.G., Logroschino, G., McCullough, M.L., Thun, M.J., et al., 2011b. Smoking and risk of amyotrophic lateral sclerosis: a pooled analysis of 5 prospective cohorts. *Arch. Neurol.* 68, 207–213.
- Wang, M.D., Gomes, J., Cashman, N.R., Little, J., Krewski, D., 2014a. 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. *PLoS One* 9, e105534.
- Wang, M.D., Gomes, J., Cashman, N.R., Little, J., Krewski, D., 2014b. A meta-analysis of observational studies of the association between chronic occupational exposure to lead and amyotrophic lateral sclerosis. *J. Occup. Environ. Med.* 56, 1235–1242.
- Wang, X.B., Cui, N.H., Gao, J.J., Qiu, X.P., Zheng, F., 2014c. SMN1 duplications contribute to sporadic amyotrophic lateral sclerosis susceptibility: evidence from a meta-analysis. *J. Neurol. Sci.* 340, 63–68.
- Waring, S.C., Esteban-Santillan, C., Reed, D.M., Craig, U., Labarthe, D.R., Petersen, R.C., et al., 2004. Incidence of amyotrophic lateral sclerosis and of the parkinsonism-dementia complex of Guam, 1950–1989. *Neuroepidemiology* 23, 192–200.
- Weisskopf, M.G., McCullough, M.L., Calle, E.E., Thun, M.J., Cudkovic, M., Ascherio, A., 2004. Prospective study of cigarette smoking and amyotrophic lateral sclerosis. *Am. J. Epidemiol.* 160, 26–33.
- Weisskopf, M.G., McCullough, M.L., Morozova, N., Calle, E.E., Thun, M.J., Ascherio, A., 2005a. Prospective study of occupation and amyotrophic lateral sclerosis mortality. *Am. J. Epidemiol.* 162, 1146–1152.
- Weisskopf, M.G., O'Reilly, E.J., McCullough, M.L., Calle, E.E., Thun, M.J., Cudkovic, M., et al., 2005b. Prospective study of military service and mortality from ALS. *Neurology* 64, 32–37.
- Weisskopf, M.G., Morozova, N., O'Reilly, E., McCullough, M.L., Calle, E.E., Thun, M.J., et al., 2009. Prospective study of chemical exposures and amyotrophic lateral sclerosis. *J. Neurol. Neurosurg. Psychiatry* 80, 558–561.
- Weisskopf, M.G., Gallo, V., O'Reilly, E.J., Vineis, P., Ascherio, A., 2010. Smoking may be considered an established risk factor for sporadic ALS. *Neurology* 74 (June 8), 8 author reply 1928–1929.
- Weisskopf, M.G., Cudkovic, M.E., Johnson, N., 2015. Military service and amyotrophic lateral sclerosis in a population-based cohort. *Epidemiology* 26, 831–838.
- Wicks, P., Ganesalingham, J., Collin, C., Prevett, M., Leigh, N.P., Al-Chalabi, A., 2007. Three soccer playing friends with simultaneous amyotrophic lateral sclerosis. *Amyotroph. Lateral Scler.* 8, 177–179.
- Wijesekera, L.C., Leigh, P.N., 2009. Amyotrophic lateral sclerosis. *Orphanet J. Rare Dis.* 4, 3.
- Wills, A.M., Cronin, S., Slowik, A., Kasperaviciute, D., Van Es, M.A., Morahan, J.M., et al., 2009. A large-scale international meta-analysis of paraonoxase gene polymorphisms in sporadic ALS. *Neurology* 73, 16–24.

- Wingo, T.S., Cutler, D.J., Yarab, N., Kelly, C.M., Glass, J.D., 2011. The heritability of amyotrophic lateral sclerosis in a clinically ascertained United States research registry. *PLoS One* 6, e27985.
- Yang, X., Xi, J., An, R., Yu, L., Lin, Z., Zhou, H., et al., 2015a. Lack of evidence for an association between the V393A variant of COQ2 and amyotrophic lateral sclerosis in a Han Chinese population. *Neurol. Sci.* 36, 1211–1215.
- Yang, X., Xi, J., Zhao, Q., Jia, H., An, R., Liu, Z., et al., 2015b. Association of the COQ2 V393A variant with parkinson's disease: a case-Control study and meta-Analysis. *PLoS One* 10, e0130970.
- Yoshida, S., Uebayashi, Y., Kihira, T., Kohmoto, J., Wakayama, I., Taguchi, S., et al., 1998. Epidemiology of motor neuron disease in the Kii Peninsula of Japan, 1989–1993: active or disappearing focus? *J. Neurol. Sci.* 155, 146–155.
- Zetterberg, H., Jacobsson, J., Rosengren, L., Blennow, K., Andersen, P.M., 2008. Association of APOE with age at onset of sporadic amyotrophic lateral sclerosis. *J. Neurol. Sci.* 273, 67–69.
- Zhang, C., 1991. The report of organophosphorus pesticides cause delayed nervous system diseases (143 cases). *Zhonghua Shen Jing Shen Ke Za Zhi* 24 (336,8, 383).
- Zhao, Q., Yang, X., Tian, S., An, R., Zheng, J., Xu, Y., 2016. Association of the COQ2 V393A variant with risk of multiple system atrophy in East Asians: a case-control study and meta-analysis of the literature. *Neurol. Sci.* 37, 423–430.
- Zhou, H., Chen, G., Chen, C., Yu, Y., Xu, Z., 2012. Association between extremely low-frequency electromagnetic fields occupations and amyotrophic lateral sclerosis: a meta-analysis. *PLoS One* 7, e48354.
- Zhou, Z., Zhang, X., Cui, F., Liu, R., Dong, Z., Wang, X., et al., 2014. Subacute motor neuron hyperexcitability with mercury poisoning: a case series and literature review. *Eur. Neurol.* 72, 218–222.