Neuro-epidemiology

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

Lazaros Belbasis[a]   Vanesa Bellou[a]   Evangelos Evangelou[a, b]

[a]Department of Hygiene and Epidemiology, University of Ioannina Medical School, Ioannina, Greece;
[b]Department of Biostatistics and Epidemiology, Imperial College London, London, UK

Abstract

Background: The pathogenesis of amyotrophic lateral sclerosis (ALS) involves both environmental and genetic factors. Our study aimed at summarising the environmental risk factors for ALS, assessing the evidence for diverse biases, and pinpointing risk factors with high epidemiological credibility. ***Methods:*** We searched PubMed from inception to August 20, 2015, to identify systematic reviews and meta-analyses of observational studies examining associations between environmental factors and ALS. For each meta-analysis, we estimated the summary effect size by the use of random-effects and fixed-effects models, the 95% CI, the 95% prediction interval (PI), and the between-study heterogeneity. We assessed the evidence of small-study effects and excess significance bias. ***Results:*** Sixteen unique meta-analyses of different risk factors and ALS were considered. Of them, 5 were statistically significant at p < 0.001 under the random-effects model. Only one factor presented robust evidence for a convincing association. This association pertained to chronic oc-cupational exposure to lead (random-effects OR 1.81, 95% CI 1.39–2.35). ***Conclusions:*** A small number of published meta-analyses on environmental factors and risk of ALS was identified, a phenomenon that could be attributed to the challenges in studying a rare neurological disease. More observational studies with adequate sample size and study design are needed to clarify the environmental component of ALS pathogenesis.

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Introduction

Amyotrophic lateral sclerosis (ALS) is a rapidly progressive, neurodegenerative disorder that affects motor neurons in brain, brainstem and spinal cord [1]. A majority of ALS cases are sporadic with unknown aetiology, whereas about 10% are familial with dominant inheritance [2]. In Europe, the incidence of ALS is about 2 per 100,000 [3], the prevalence of ALS is 5.40 per 100,000 [4] and the overall lifetime risk of developing ALS is 1:350 for men and 1:400 for women [5]. Although there is a clear genetic predisposition to ALS [1], environmental factors are likely to be involved in the pathogenesis of the disease.

Dr. Evangelos Evangelou, PhD
Department of Hygiene and Epidemiology
University of Ioannina Medical School
GR–45110 Ioannina (Greece)
E-Mail vangelis@cc.uoi.gr

Environmental exposures proposed to be linked with ALS include exposure to lead and other heavy metals, and pesticides, while medical conditions including head trauma have also been associated with ALS [1]. Clarifying the environmental component of ALS etiology could lead to better understanding of disease pathophysiology. Due to rapid accumulation of observational studies, systematic reviews accompanied by a quantitative synthesis of the available evidence are the most appropriate approaches to identify credible associations and to systematically appraise the observational studies.

Previous umbrella reviews of systematic reviews and meta-analyses that assessed environmental factors associated with Parkinson's disease and multiple sclerosis revealed several caveats pertaining to large statistical between-study heterogeneity, small sample sizes, and small-study effects and other biases [6, 7]. Out of 44 associations, only 3 showed strong evidence for association with multiple sclerosis [6], whereas for Parkinson's disease, only 2 of the examined environmental factors achieved top ranking in the assessment of the meta-analyses [7]. All these methodological issues introduce doubts for the epidemiological credibility of several associations between the environmental factors and the aforementioned neurological diseases.

To further expand the mapping and the critical appraisal of environmental risk factors across additional neurological conditions, we apply similar methodology for ALS. Our aim is to provide an overview of the range and validity of the published associations of diverse environmental risk factors with ALS as examined in published systematic reviews and meta-analyses, and to evaluate the existence and the extent of potential biases in this literature. Finally, we highlight which of the previously studied associations that have been synthesized with meta-analyses have the strongest evidence for association.

Methods

Search Strategy and Eligibility Criteria

We conducted an umbrella review, a systematic collection and assessment of multiple systematic reviews and meta-analyses performed on a specific research topic [8]. The methods of the umbrella review are standardized and in this work we follow state-of-the-art approaches as previously published umbrella reviews on risk factors for various outcomes including multiple sclerosis and Parkinson's disease [6, 7]. Specifically, our approach includes a comprehensive and systematic literature search, application of state-of-the art methods for the evaluation of potential biases, and assessment of the epidemiological credibility of the examined associations by predefined criteria.

We systematically searched PubMed from inception to August 20, 2015 to identify systematic reviews and meta-analyses of observational studies examining associations between environmental (non-genetic) factors and ALS. The search strategy used the keywords ('amyotrophic lateral sclerosis' OR 'motor neuron disease') AND ('systematic review' OR 'meta-analysis'). The full text of potentially eligible articles was scrutinised independently by 2 investigators (L.B. and V.B.). We excluded meta-analyses that investigated the association between genetic polymorphisms and risk of ALS because these factors are already summarized and evaluated in ALS Gene (http://www.alsgene.org/) [9]. We excluded meta-analyses examining environmental risk factors for other motor neuron diseases than ALS. We also excluded meta-analyses that examined ALS as a risk factor for other medical conditions. We did not apply any language restrictions in the selection of eligible studies. When more than one meta-analysis on the same research question was eligible, the meta-analysis with the largest number of component studies with data on individual studies' effect sizes was retained for the main analysis. We enhanced the literature search by accessing additional databases (the Cochrane Library and EMBASE).

Data Extraction

Data extraction was conducted by 2 independent investigators (L.B. and V.B.). In case of discrepancies, the final decision was made by a third investigator (E.E.). From each eligible article, we extracted information on the first author, journal, year of publication, examined risk factors, and number of studies included. We also recorded the number of cases and controls in each study for each risk factor. When the sample sizes of the primary studies were not reported in the article of meta-analysis, we retrieved the published report of the primary study and we extracted the relevant data. If a quantitative synthesis was performed, we also recorded the study-specific relative risk estimates (risk ratio (RR), odds radio (OR), hazard ratio (HR)) along with the corresponding CI.

Statistical Analysis

For each meta-analysis, we estimated the summary effect size and its 95% CI with both fixed-effects and random-effects models [10, 11]. We also estimated the 95% PI, which further accounts for between-study heterogeneity and evaluated the uncertainty for the effect that would be expected in a new study addressing that same association [12, 13]. In the case of meta-analyses with continuous data, the effect estimate was transformed to an OR with an established formula [14]. For the largest study of each meta-analysis, we estimated the SE of the effect size and examined whether the SE was less than 0.10. In a study with an SE of less than 0.10, the difference between the effect estimate and the upper or lower 95% CI is less than 0.20 (i.e. this uncertainty is less than what is considered a small effect size).

Between-study heterogeneity was assessed and quantified by the I^2 metric [15]. I^2 ranges between 0 and 100% and describes the percentage of total variation across studies that is due to heterogeneity rather than chance [16]. Values exceeding 50 or 75% are usually judged to represent large or very large heterogeneity, respectively.

We applied the regression asymmetry test proposed by Egger and colleagues [17] in order to assess whether there was evidence for small-study effects (i.e. whether smaller studies tend to give substantially larger estimates of effect size compared with larger
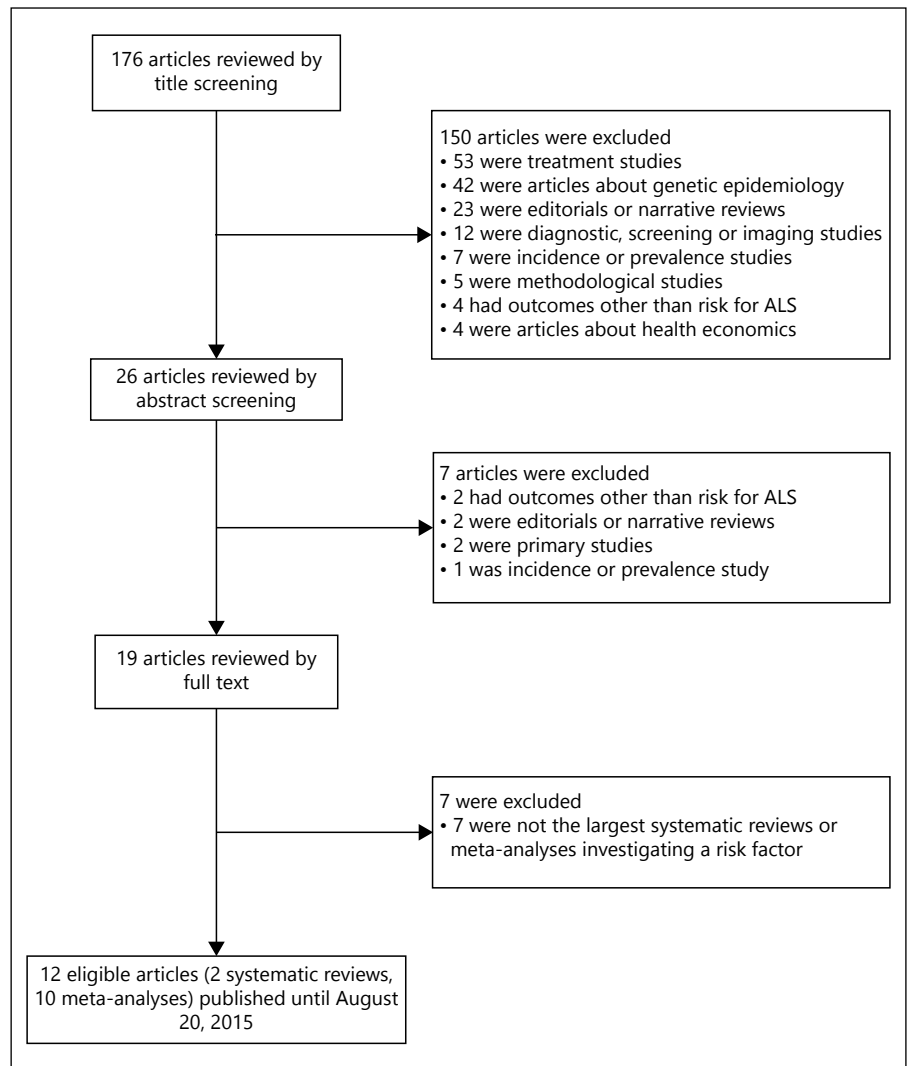
176 articles reviewed by title screening

150 articles were excluded
• 53 were treatment studies
• 42 were articles about genetic epidemiology
• 23 were editorials or narrative reviews
• 12 were diagnostic, screening or imaging studies
• 7 were incidence or prevalence studies
• 5 were methodological studies
• 4 had outcomes other than risk for ALS
• 4 were articles about health economics

26 articles reviewed by abstract screening

7 articles were excluded
• 2 had outcomes other than risk for ALS
• 2 were editorials or narrative reviews
• 2 were primary studies
• 1 was incidence or prevalence study

19 articles reviewed by full text

7 were excluded
• 7 were not the largest systematic reviews or meta-analyses investigating a risk factor

12 eligible articles (2 systematic reviews, 10 meta-analyses) published until August 20, 2015

Fig. 1. Flowchart of literature search for systematic reviews and meta-analyses published from inception until August 20, 2015.

studies). A p < 0.10 accompanied by a more conservative effect in larger studies than in random-effects meta-analysis was judged to be evidence for the existence of small-study effects.

We also applied the excess statistical significance test, which evaluates whether the observed (O) number of studies with nominally statistically significant results ('positive' studies, p < 0.05) is larger than their expected (E) number [18]. E is calculated in each meta-analysis by the sum of the statistical power estimates for each component study. The true effect size for any meta-analysis is not known. We estimated the power of each component study using the effect size of the largest study (smallest SE) in a meta-analysis [19]. The power of each study was calculated using a non-central t distribution [20]. Excess statistical significance for single meta-analyses was claimed at 2-sided p < 0.10 with O > E as previously proposed.

Finally, we identified and reported the associations that had the strongest validity and were not suggestive of bias. Specifically, we characterized as convincing the associations that met the following criteria: had significance according to random-effects meta-analysis less than 0.001 [21, 22]; were based on greater than 1,000 cases; had between-study heterogeneity that was not large (I^2 <50%) and a 95% PI excluded the null value; and had not evidence of small-study effects and excess significance bias. We characterized as suggestive the associations that presented not large between-study heterogeneity, and had not evidence of small-study effects and excess significance bias. The remaining significant associations were characterized as weak.

The statistical analysis and the power calculations were performed with STATA version 12.0.

Results

Our literature search on PubMed yielded 176 articles, and 12 articles were deemed eligible (fig. 1). The additional literature search on the Cochrane Library and

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EMBASE did not result in any further eligible articles. Two systematic reviews [23, 24] did not quantify the existent evidence, while 10 articles additionally performed a meta-analysis. These articles corresponded to 16 unique meta-analyses, examining 16 environmental factors and including 138 primary observational studies in total. One paper [25] included 2 meta-analyses on the association between smoking and ALS, providing analyses by 2 different level of comparisons (ever smokers vs. never smokers, and current smokers vs. never smokers). In our study, we included the meta-analysis that compared the difference in risk between ever smokers and never smokers, as this analysis included more ALS cases and more observational studies. Seven of the 19 articles screened by full text were excluded because a larger meta-analysis that examined the same risk factors was found. These articles pertained to extremely-low-frequency electromagnetic fields [26], smoking [27], pesticides [28–30], and heavy metals [29, 31].

The 2 systematic reviews of observational studies without further quantitative synthesis examined physical activity [24] (37 studies) and diabetes mellitus [23] (7 studies) as risk factors for ALS. In both cases, the authors concluded that the evidence is limited and quite heterogeneous, and could not be used to draw definite conclusions. In both systematic reviews, the authors mentioned that a quantitative synthesis was not attempted because of heterogeneity in study designs, in ascertainment and definition of the exposure.

The median number of studies per meta-analysis was 8 (IQR 5–9) and the median number of cases was 1,779 (IQR 937–2,911). The 16 meta-analyses covered a range of risk factors, classified as biomarkers (n = 5), dietary factors (n = 2), exposure to toxic agents (n = 5) and medical history (n = 2). The number of cases was greater than 1,000 in 11 meta-analyses. All eligible meta-analyses used summary-level data from published literature and none of them had access to individual participant data.

Nine of 16 meta-analyses reported effects that were significant at $p < 0.05$ under the random-effects model. Five were significant at $p < 0.001$ under the random-effects model (table 1): farming [32], lead [33], n-3 fatty acids [34], pesticides [32] and serum uric acid [35]. The heterogeneity was large ($I^2 \geq 50$ and $\leq 75\%$) in 5 meta-analyses and very large ($I^2 > 75\%$) in 3 meta-analyses. The meta-analyses with very large heterogeneity examined serum total cholesterol [36], serum HDL [36] and serum LDL [36]. Only 2 meta-analyses had a 95% PI excluding the null value, pertaining to lead and n-3 fatty acids. In 8 of the eligible meta-analyses, the

SE of the largest study was less than 0.10 in a log OR scale.

Two meta-analyses presented statistically significant Egger's test ($p < 0.10$). In one of these 2 meta-analyses, the largest individual study had a more conservative effect size than the random-effects summary effect size, indicating the presence of small-study effects. Assuming that the effect size in the largest study was the true effect, 5 of the 16 meta-analyses had a significant difference ($p < 0.10$) between the number of observed and expected positive studies (table 2). These meta-analyses pertained to serum total cholesterol, serum HDL, serum LDL, statins and pesticides.

By applying our credibility criteria, 7 out of the 16 eligible environmental factors presented a non-significant association ($p > 0.05$). Of the 9 remaining significant associations at $p < 0.05$, 2 associations (extremely low-frequency electromagnetic fields, serum uric acid) presented large heterogeneity and one association (n-3 fatty-acids intake) was significant for small-study effects and an additional association (pesticides) presented hints for excess statistical significance. Thus, the evidence of association for those 4 factors was weak. Of the remaining 5 associations, 4 (other heavy metals, farming, head injury, β-carotene) had 95% PI including the null value, whereas one association (other heavy metals) included less than 1,000 cases. These 4 associations were supported by suggestive evidence.

Finally, only one of the 16 meta-analyses (exposure to lead [33]) presented convincing evidence for an association with ALS, supported by more than 1,000 cases, $p < 0.001$ under the random-effects model, small or moderate heterogeneity ($I^2 < 50\%$), a 95% PI excluding the null value, absence of evidence for small-study effects and excess statistical significance. This association had a summary OR of 1.81 (95% CI 1.39–2.35; $p = 2.13 \times 10^{-6}$) with small heterogeneity ($I^2 = 12.7\%$). Evidence was supported by a total of 1,228 cases. An overall assessment of significant risk factors for ALS, using the predefined criteria based on the sample size, the level of replication, a more conservative threshold for $p < 0.001$, and the presence of small-study effects, is presented in table 3.

Discussion

We present an overview and critical appraisal of environmental risk factors that have been associated with ALS in published systematic reviews and meta-analyses, by applying specific methodological criteria. To our knowl-

Table 1. Characteristics and quantitative synthesis of the 16 eligible meta-analyses of environmental risk factors for ALS

Reference	Risk factor	Level of comparison	Total number of cases/controls	Number of studies	Effect size	Random-effects summary effect size (95% CI)	p random	95% PI	Fixed-effects summary effect size (95% CI)	p fixed
Biomarkers										
Abraham et al. [35], 2014	Serum uric acid	High values vs. low values	311/515	3	OR*	0.31 (0.18–0.52)	1.27×10^{-5}	0.001–1.37	0.34 (0.26–0.45)	3.94×10^{-15}
Huang et al. [36], 2015	Serum HDL	High values vs. low values	1,930/3,635	8	OR*	1.05 (0.75–1.46)	7.94×10^{-1}	0.33–3.27	0.97 (0.87–1.08)	5.56×10^{-1}
Huang et al. [36], 2015	Serum LDL	High values vs. low values	1,930/3,635	8	OR*	0.95 (0.44–2.05)	9.02×10^{-1}	0.06–15.85	0.97 (0.86–1.08)	5.41×10^{-1}
Huang et al. [36], 2015	Serum total cholesterol	High values vs. low values	1,930/3,635	8	OR*	0.99 (0.59–1.68)	9.96×10^{-1}	0.15–6.49	1.06 (0.95–1.19)	2.81×10^{-1}
Huang et al. [36], 2015	Serum triglycerides	High values vs. low values	1,627/1,535	6	OR*	0.98 (0.82–1.17)	8.37×10^{-1}	0.64–1.51	0.98 (0.86–1.11)	7.52×10^{-1}
Dietary factors										
Fitzgerald et al. [53], 2013	β-Carotene intake	2,500 µg/day increase	1,093/1,052,482	9	RR	0.92 (0.87–0.97)	2.24×10^{-3}	0.86–0.98	0.92 (0.87–0.97)	2.24×10^{-3}
Fitzgerald et al. [34], 2014	n-3 fatty acids intake	1 g/day increase	994/1,055,843	8	RR	0.71 (0.59–0.85)	1.95×10^{-4}	0.49–1.01	0.74 (0.64–0.86)	9.35×10^{-5}
Medical history										
Chen et al. [42], 2007	Head injury	Exposed vs. not exposed	2,563/3,977	8	OR	1.65 (1.09–2.51)	1.80×10^{-2}	0.59–4.64	1.66 (1.26–2.18)	2.76×10^{-4}
Zheng et al. [54], 2013	Statins	Exposed vs. not exposed	765/128,405	3	RR	1.15 (0.78–1.70)	4.85×10^{-1}	0.01–1.06	1.10 (0.92–1.30)	3.01×10^{-1}
Exposure to environmental agents										
Alonso et al. [25], 2010	Smoking	Ever smokers vs. never smokers	3,958/3,016,341	20	RR	1.12 (0.98–1.27)	1.03×10^{-1}	0.70–1.77	1.13 (1.05–1.22)	2.00×10^{-3}
Kang et al. [32], 2014	Farming	Exposed vs. not exposed	9,338/2,605,321	10	OR	1.42 (1.17–1.73)	3.75×10^{-4}	0.90–2.26	1.38 (1.24–1.54)	1.16×10^{-8}
Kang et al. [32], 2014	Pesticides	Exposed vs. not exposed	9,534/3,716,144	15	OR	1.44 (1.22–1.70)	2.04×10^{-5}	0.94–2.20	1.38 (1.25–1.52)	1.64×10^{-10}
Kang et al. [32], 2014	Rural living	Exposed vs. not exposed	559/1,032	5	OR	1.25 (0.84–1.88)	2.76×10^{-1}	0.35–4.54	1.19 (0.93–1.51)	1.63×10^{-1}
Wang et al. [33], 2014	Other heavy metals	Exposed vs. not exposed	457/558	4	OR	2.13 (1.33–3.41)	1.60×10^{-3}	0.48–9.52	1.98 (1.37–2.85)	2.43×10^{-4}
Wang et al. [33], 2014	Lead	Exposed vs. not exposed	1,228/1,544	9	OR	1.81 (1.39–2.35)	8.72×10^{-6}	1.14–2.88	1.79 (1.41–2.27)	2.13×10^{-6}
Zhou et al. [55], 2012	Extremely low-frequency electromagnetic fields	Exposed vs. not exposed	12,681/9,902,859	17	OR	1.29 (1.03–1.62)	3.00×10^{-2}	0.64–2.59	1.12 (1.00–1.25)	4.80×10^{-2}

* Weighted mean difference transformed to OR.

Table 2. Bias assessment of the 16 eligible meta-analyses of environmental risk factors for ALS

Reference	Risk factor	Effect size	Largest study effect size (95% CI)	SE	I^2	Egger test p value	Observed significant studies	Expected significant studies	Excess significance test p value
Biomarkers									
Abraham et al. [35], 2014	Serum uric acid	OR*	0.48 (0.33–0.69)	0.187	71.9	0.117	3	2.51	0.45
Huang et al. [36], 2015	Serum HDL	OR*	0.97 (0.79–1.18)	0.099	87	0.548	3	0.44	8.00×10^{-5}
Huang et al. [36], 2015	Serum LDL	OR*	1.15 (0.94–1.40)	0.100	97.6	0.862	4	1.11	3.18×10^{-3}
Huang et al. [36], 2015	Serum total cholesterol	OR*	1.12 (0.92–1.36)	0.099	94.8	0.772	4	0.88	4.10×10^{-4}
Huang et al. [36], 2015	Serum triglycerides	OR*	0.91 (0.75–1.11)	0.099	35.1	0.844	0	0.56	NP
Dietary factors									
Fitzgerald et al. [53], 2013	β-Carotene intake	RR	0.91 (0.86–0.98)	0.033	0	0.917	1	0.60	0.59
Fitzgerald et al. [34], 2014	n-3 fatty acids intake	RR	0.76 (0.50–1.15)	0.215	0	0.056[†]	1	2.32	NP
Medical history									
Chen et al. [42], 2007	Head injury	OR	1.60 (1.00–2.40)	0.223	42.6	0.751	2	5.57	NP
Zheng et al. [54], 2013	Statins	RR	0.98 (0.79–1.21)	0.109	74.2	0.767	1	0.16	0.03
Exposure to environmental agents									
Alonso et al. [25], 2010	Smoking	RR	1.37 (1.17–1.60)	0.080	57.6	0.734	5	8.84	NP
Kang et al. [32], 2014	Farming	OR	1.20 (1.02–1.41)	0.083	42.1	0.857	5	3.26	0.24
Kang et al. [32], 2014	Pesticides	OR	1.20 (1.02–1.41)	0.083	41.2	0.163	7	4.03	0.08
Kang et al. [32], 2014	Rural living	OR	0.80 (0.60–1.30)	0.262	59.7	0.618	2	0.89	0.19
Wang et al. [33], 2014	Other heavy metals	OR	1.52 (0.95–2.42)	0.239	26	0.368	1	1.73	NP
Wang et al. [33], 2014	Lead	OR	1.90 (1.14–3.17)	0.261	12.7	0.110	3	6.13	NP
Zhou et al. [55], 2012	Extremely low-frequency electromagnetic fields	OR	1.30 (1.10–1.60)	0.096	58.9	0.034	5	7.14	NP

NP = Not pertinent (because the number of expected significant studies was larger than the number of observed significant studies).

* Weighted mean difference transformed to OR.

[†] In the annotated paper, the Egger test was statistically significant (p < 0.10) and the largest study has more conservative effect size compared to the summary effect size under random effects, denoting the existence of small-study effects.

Table 3. Assessment of epidemiological credibility across the 16 associations of environmental risk factors with ALS

Risk factors	Sample size (number of cases)	Significance threshold (under the random-effects model)	95% PI rule	Estimate of heterogeneity	Small-study effects or excess significance bias	Random-effects summary effects size (95% CI)
Associations supported by convincing evidence						
Lead [33]	>1,000	<0.001	Excluding the null value	Not large	None	1.81 (1.39–2.35)
Associations supported by suggestive evidence						
Farming exposure [32]	>1,000	<0.001	Including the null value	Not large	None	1.42 (1.17–1.73)
Other heavy metals [33]	<500	<0.05 but >0.001	Including the null value	Not large	None	2.13 (1.33–3.41)
β-Carotene intake [53]	>1,000	<0.05 but >0.001	Including the null value	Not large	None	0.92 (0.87–0.97)
Head injury [42]	>1,000	<0.05 but >0.001	Including the null value	Not large	None	1.65 (1.09–2.50)
Associations supported by weak evidence						
n-3 fatty acids intake [34]	<1,000 but >500	<0.001	Excluding the null value	Not large	Small-study effects	0.66 (0.53–0.82)
Extremely low-frequency electro- magnetic fields [55]	>1,000	<0.05 but >0.001	Including the null value	Large	None	1.29 (1.03–1.62)
Pesticides [32]	>1,000	<0.001	Including the null value	Not large	Excess significance bias	1.44 (1.22–1.70)
Serum uric acid [35]	<500	<0.001	Including the null value	Large	None	0.31 (0.18–0.52)
Non-significant associations						
Rural living [32]	<1,000 but >500	>0.05	Including the null value	Large	None	1.25 (0.84–1.88)
Serum total cholesterol [36]	>1,000	>0.05	Including the null value	Very large	Excess significance bias	0.99 (0.59–1.68)
Serum HDL [36]	>1,000	>0.05	Including the null value	Very large	Excess significance bias	1.05 (0.75–1.46)
Serum LDL [36]	>1,000	>0.05	Including the null value	Very large	Excess significance bias	0.95 (0.44–2.05)
Serum triglycerides [36]	>1,000	>0.05	Including the null value	Not large	None	0.98 (0.82–1.17)
Statins [54]	<1,000 but >500	>0.05	Including the null value	Large	Excess significance bias	1.15 (0.78–1.70)
Smoking [25]	>1,000	>0.05	Including the null value	Large	None	1.12 (0.98–1.27)

edge, this is the first attempt to summarize the evidence from existing meta-analyses on environmental factors for ALS, and their limitations. Juxtaposing other umbrella reviews [6, 37] and field synopses of genetic associations of complex diseases [38], an assessment of environmental factors, especially for rare diseases, could be rather challenging due to biases derived from the study design, small sample sizes, and misclassification problems mainly for the exposure and so on. A stringent, comprehensive appraisal of current evidence is necessary to minimize false positive associations.

In our study, we observed a small number of published meta-analyses synthesizing the evidence on environmental factors and risk of ALS. This could be attributed to the small number of observational studies due to the difficulties of conducting research focusing on a rare neurological disease (e.g. the vast number of environmental exposures that can be studied, the large cost of conducting population-based studies combined with small interest of funding bodies for a rare disease) [5]. Overall, 16 risk factors have been studied in a meta-analysis for an association with the disease, including biomarkers, dietary factors, exposure to toxic agents and medical history. However, only one of these factors, chronic occupational exposure to lead, was supported by strong epidemiological credibility, as expressed by large sample size, not large heterogeneity, absence of small-study effects and excess statistical significance, a p < 0.001, and a 95% PI excluding the null value.

The effect size and the magnitude of the association between chronic occupational exposure to lead and risk for ALS were strong. Indeed, the predominant theory on the environmental counterpart to ALS pathogenesis is based on lead toxicity [1]. In humans, the classic form of lead neuropathy is characterized by ALS-like clinical manifestations, such as weakness initially involving the wrist and finger extensors and with spread to other muscles [33]. Additionally, several studies measured biomarkers of lead exposure in ALS patients and they reported higher levels of lead in blood for ALS patients compared to healthy controls [39, 40]. However, the observational studies on the association between lead exposure and risk for ALS included a self-reported assessment of occupational exposure to lead [41]. To clarify the association between lead and risk of ALS, it is apparent that more epidemiological studies are needed to assess the exposure to lead by biomarkers on biological fluids. Exposure to other heavy metals, such as mercury and aluminum that has also been proposed to be associated with risk for ALS [1], was supported by suggestive evidence,

indicating that there is no solid body of evidence to underpin this association.

Furthermore, head injury presented a positive association with ALS, supported by suggestive evidence and a p value close to nominal significance threshold of 0.05. This association has been argued to have biological plausibility, and several mechanisms, that apply to other neurological diseases as well, have been proposed to explain the neurological damage induced by head injury [42, 43]. However, as in the case of Parkinson's disease [7, 44], the association between head injury and ALS may be attributed to inverse causation, and it may be perceived as a prodromal manifestation of ALS. To draw a definite conclusion on the relation between head injury and ALS, we should take into account the time lap between the event of head injury and the clinical manifestation or diagnosis of ALS. Although the largest study reported a statistically significant effect, the observational studies were characterized by poor reporting on the assessment of exposure, small sample sizes and low methodological quality [42, 45], while they did not perform adequate adjustments for potential confounders. The low statistical power and the existence of potential confounders introduce doubts for the credibility of the association between head injury and ALS.

As expected, a majority of meta-analyses on the association of biomarkers with risk for ALS had large or very large between-study heterogeneity and, in several cases, hints suggestive for excess statistical significance. A similar picture was also observed in the field of multiple sclerosis [6] and Parkinson's disease [7]. The identification of credible biomarkers for ALS is essential for diagnosis and prognosis of ALS, and the development of novel biomarkers could even alter the design of clinical trials in the field of ALS [3]. However, the respondent meta-analyses presented evidence for various statistical biases, verifying the absence of any plausible biomarker. Previous research in other fields highlighted that the lack of replication, the large uncertainty in the effect sizes and the existence of statistical biases are a common phenomenon in the literature of biomarkers, indicating the poor design of the observational studies [46, 47].

Regarding the analysis of bias, one third of the examined meta-analyses were characterized by the presence of excess statistical significance. Compared to the previously published umbrella reviews, the level of excess statistical significance is higher than the case of multiple sclerosis but quite smaller than the case of Parkinson's disease [6, 7]. Also, a large proportion of the examined meta-analyses was poorly replicated, given that half of the me-

ta-analyses had large or very large heterogeneity, and only 2 of the meta-analyses presented a 95% PI excluding the null value. The level of replication is comparable with that in the case of multiple sclerosis and Parkinson's disease [6, 7]. Heterogeneity might often be a manifestation of bias in some studies of a meta-analysis, but could also emerge from genuine differences across studies. Potential sources of heterogeneity might be the mixture of cohort studies and case–control studies in some of the meta-analyses, and differences in exposure assessment.

Our analysis has some caveats. First, both asymmetry and excess statistical significance tests offer suggestions of bias, and not definitive proof thereof. Second, effect inflation might affect even the results of the largest studies because often these studies do not have an adequate sample size and appropriate study design or might have had inherent biases themselves. Thus, our estimates of the extent of excess statistical significance are probably conservative. Additionally, we did not appraise the quality of the individual component primary studies because this was beyond the scope of this umbrella review. This was the aim of the original systematic reviews and meta-analyses, which should include an assessment of study quality and examine whether the study should be included in the quantitative calculations. Also, in our analysis, we assessed only associations considered by meta-analyses of observational studies. Thus, we might miss other associations supported by adequate evidence that have not yet been assessed through meta-analytic approaches.

Acknowledging these caveats, our assessment depicts the status of evidence on 16 associations between environmental factors and risk for ALS. Only chronic occupational exposure to lead provided credible evidence for positive association with ALS without the presence of substantial caveats. Our approach could be complemented by the application of existing evaluation tools focused on the methodological appraisal of systematic reviews, such as AMSTAR [48, 49]. Data from more studies and investigation of sources of heterogeneity are needed to better understand the associations between the remaining risk factors and ALS. As previously suggested, for observational research and biomarker studies in general, use of standardized definitions for outcomes and exposures, adoption of reporting guidelines (e.g. Strengthening the Reporting of Observational Studies in Epidemiology (STROBE)) [50], and registration of hypothesis-testing observational studies might help to improve the evidence in the future [51, 52].

Author Contributions

L.B. and V.B. had the original idea for the manuscript and all authors contributed to design the study. L.B., V.B. performed the analyses and all authors interpreted the results. L.B., V.B. wrote the first draft of the manuscript. All authors critically reviewed, wrote and approved the final version.

Disclosure Statement

The authors declare that there are no conflicts of interest.

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