Appendix for:

Emerging insights into the complex genetics and pathophysiology of ALS

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Concordant and discordant ALS exposome studies

Several studies have been launched to define the ALS exposome; importantly, not all studies are concordant. A case-control (n=156; n=128) study conducted in Michigan found two organochlorine pesticides, two polychlorinated biphenyls, and one brominated flame retardant associate with ALS risk in multivariable modeling.[1] Environmental risk scores (ERS) were generated based on blood levels of pesticides and organic pollutants in another Michigan ALS cohort (n=167); the highest ERS quartile had an OR 2·70 for mortality versus the lowest quartile, after adjusting for age at diagnosis, sex, and other covariates.[2] Pollutants that contribute significantly to the ERS include polychlorinated biphenyls, polybrominated diphenyl ethers, and a dichlorodiphenyldichloroethylene. In contrast, cerebrospinal fluid analysis from 38 ALS versus 38 control participants from Italy did not find differences in organochlorine pesticides, polychlorinated biphenyls, or polycyclic aromatic hydrocarbons.[3] Exposure duration is an important consideration in exposome research, as dictated by the gene-time-environment hypothesis. Occupational pesticide exposure is routinely linked to ALS, as seen in a recent meta-analysis.[4] A recent Italian study found that occupational history in the agricultural sector only correlated with ALS risk in individuals with an agricultural occupational history longer than 10 years (OR 2·72, 95%CI 1·02-7·20).[5] There was no association in individuals with a shorter occupational history.

Metals are an additional candidate risk for ALS, but again some studies are discordant.[4] Analysis of the Danish National Patient Registry did not find an association between metal occupational exposure history for chromium, iron, and nickel with ALS risk.[6] However, other studies report risk, such as analysis of banked blood samples from participants that eventually developed ALS (n=107) from the European Prospective Investigation into Cancer and Nutrition cohort.[7] Blood cadmium (OR 2·04, 95%CI 1·08-3·87) and, to a lesser extent, lead (OR 1·89, 95%CI 0·97-3·67) in presymptomatic individuals correlates with future ALS risk. Teeth were analyzed from ALS participants (n=36) versus controls (n=31) using laser ablation-inductively coupled plasma-mass spectrometry to assess metal uptake over lifetime.[8] Exposures linked to ALS at distinct time points with various metals, chromium (at 15 years), manganese (at birth), nickel (at 8 years), tin (at 2 years), and zinc (at 6 years).

Discordance across exposome studies in ALS may arise from population size or characteristics (*e.g.*, location, genetics), exposure duration, time of exposure, adjustment parameters, and methodology (*e.g.*, historical exposure estimation versus analyte measurements, sample tissue type). Thus, despite a significant body of work and identified ALS-environmental links, large prospective cohort studies are needed.[9] These will require detailed registries of patient medical information linked to personal-level data and occupational and residential history with banked biosamples. Studies should evaluate how the exposome modifies disease progression and outcomes,[2] as well as onset risk. Furthermore, environmental, residential, and occupational risks may not be geographically uniform, necessitating large prospective cohorts across diverse regions, possibly globally. Additionally, geographically distinct populations may also be genetically distinct, which could modify their exposure risk. Although gene-environment interaction studies have been conducted for single gene candidates,[10] multi-Omics studies will be needed that bridge genetics,[11] *i.e.*, mono-, oligo- and polygenic risk, with exposome, *i.e.*, ERS, to truly comprehend ALS risk and progression.

Table. Environmental studies in ALS. English language studies of primary literature published in the past 5 years were identified with the search terms of ALS combined with environment, exposure, cluster, metals, pesticides, pollutant and were selected to show the range of exposure assessments and based on population size, publication impact, and use of novel techniques.

Publication	Exposure	Study location and population	Year(s)	Study design	Outcome
Dickerson[12]	Occupational diesel exposure	Danish National Patient Registry; 1,639 ALS; 151,975 controls	1982-2013	Case-control	Diesel exhaust linked to ALS risk in males only (OR 1·20, 95%CI 1·05-1·38)
Dickerson[6]	Occupational metal exposures	Danish National Patient Registry	1982-2013	Case-control	No association with ALS risk
Figueroa-Romero [8]	Lifetime metal exposure in teeth	Michigan, United States; 36 ALS; 31 controls	Lifetime	Case-control	Metals higher in ALS vs controls: 1·49x chromium (1·11-1·82; at 15 years), 1·82x manganese (1·34-2·46; at birth), 1·65x nickel (1·22-2·01; at 8 years), 2·46x tin (1·65-3·30; at 2 years), 2·46x zinc (1·49-3·67; at 6 years)
Filippini[13]	Clinical and lifestyle factors	Italy; 95 ALS; 135 controls	2008–2011	Case-control	Trauma/head trauma (OR 2·61, 95%CI 1·19-5·72), electric shock (OR 2·09, 95%CI 0·62-7·06), private well drinking water (OR 1·38, 95%CI 0·73-2·27), herbicide gardening use (OR 1v95, 95%CI 0·88-2·27), overall fish consumption (OR 0·27, 95%CI 0·12-0·60)

Filippini[5]	Occupational/ environmental exposures	Italy; 95 ALS; 135 controls	2008–2011	Case-control	Agricultural sector work >10 years (OR 2·72, 95% 1·02-7·20), occupational thinner exposure (OR 2·27, 95%CI 1·14-4·54), occupational paint remover exposure (OR 2·01, 95%CI 0·90-4·48), environmental electromagnetic field exposure (OR 2·41, 95%CI 1·13-5·12), occupational metal exposure (OR 4·20, 95%CI 1·88-9·38), residence near water body (OR 1·83, 95%CI 1·04-3·21)
Goutman[2]	Plasma persistent organic pollutants	Michigan, United States; 167 ALS	2012-2015	Prospective	Plasma pollutants with the largest contribution to environmental risk scores were PBDE 154 (HR 1·53, 95%CI 0·90-2·61), PCB 118 (HR 1·50, 95%CI 0·95-2·39), PCB 138 (HR 1·69, 95%CI 0·99-2·90), PCB 151 (HR 1·46, 95%CI 1·01-2·10), PCB 175 (HR 1·53, 95%CI 0·98-2·40), p,p'-DDE (HR 1·39, 95%CI 1·07-1·81)
Myung[14]	Air pollution	Korea; 617 ALS	2008-2014	Time-stratified case-crossover	ALS symptom exacerbation linked to IQR increase in $PM_{2.5}$ (OR 1·21, 95%CI 1·08-1·35), PM_{10} (OR 1·13, 95%CI 1·02-1·25), SO_2 (OR 1·19, 95%CI 1·01-1·41), CO (OR 1·19, 95%CI 1·03-1·36)
Peters[15]	Occupational exposures and lifestyle history	European Multidisciplinary ALS Network Identification to Cure Motor Neurone Degeneration cohort; 1,323 ALS; 2,704 controls	2010-2015	Case-control	Extremely low-frequency magnetic fields (OR 1·16, 95%CI 1·01-1·33), electric shock (OR 1·23, 95%CI 1·05-1·43)

Peters[7]	Blood metals	European Prospective Investigation into Cancer and Nutrition cohort; 107 ALS; 319 controls	1993-1999	Prospective	Presymptomatic ALS linked to blood cadmium (OR 2·04, 95%CI 1·08-3·87), lead (OR 1·89, 95%CI 0·97-3·67), zinc (OR 0·50, 95%CI 0·27-0·94)
Pupillo[16]	Trauma causing injury	European Amyotrophic lateral sclerosis cohort; 575 ALS; 1,150 controls	2008-2012	Case-control	Disabling traumatic events linked to ALS (OR 1·54, 95%CI 1·24-1·92)
Seals[17]	Cumulative occupational exposure to formaldehyde	Danish National Patient Registry; 3,650 ALS; 14,600 controls	1982-2009	Case-control	Formaldehyde exposure raises ALS risk (OR 1·3, 95%CI 1·2-1·4)
Seelen[18]	Traffic related air pollution	Netherlands, European Study of Cohorts for Air Pollution Effects cohort; 917 ALS; 2,662 controls	2006-2013	Case-control	Upper quartile exposure risks, $PM_{2.5}$ (OR 1·67, 95%CI: 1·27-2·18), NO_2 (OR 1·74, 95%CI 1·32-2·30), NO_x (OR 1·38, 95%CI 1·07-1·77)
Su[1]	Plasma persistent organic pollutants	Michigan, United States; 156 ALS; 128 controls	2011-2014	Case-control	Plasma pentachlorobenzene (OR 2·57, 95%CI 1·31-5·02), cis-chlordane (OR 6·51, 95%CI 2·05-20·73, PCB 151 (OR 1·66, 95%CI 1·03-2·67)
Vinceti[19]	Agricultural crop proximity	Italy; 703 ALS; 2737 controls	1998-2011	Case-control	Proximity to agricultural crop (OR 0·92, 95%CI 0·78-1·09)
Vinceti[20]	Magnetic fields from high-voltage power lines	Italy; 703 ALS; 2,737 controls	1998-2011	Case-control	No association with ALS risk
Vinceti[3]	Persistent organic pollutants	Italy; 38 ALS; 38 controls	1994-2013	Prospective	No association with ALS risk with OCPs, PCBs or PAHs

Visser[21]	Occupational exposures	Euro-MOTOR cohort; 1,557 ALS; 2,922 controls	2011-2014	Case-control	Silica dust (OR 1·73, 95%CI 1·28-2·33), organic dust (OR 1·33, 95%CI 1·10-1·60)
Wang[4]	Risk factors	Meta-analysis	2013-2016	Meta-analysis	Lead exposure (OR 1·72, 95%CI 1·33-2·23), heavy metals (OR 1·69, 95%CI 1·13-2·52), pesticides (OR 1·48, 95%CI 1·18-1·86), agricultural chemicals (OR 3·08, 95%CI 1·43-6·63), solvents (OR 1·43, 95%CI 1·10-1·86), prior trauma (OR 1·73, 95%CI 1·43-2·09), electric shock (OR 3·27, 95%CI 1·87-5·73)

CI, confidence interval; CO, carbon monoxide; DDE dichlorodiphenyldichloroethylene; HR, hazard ratio; IQR, interquartile range; NO, nitrogen oxide; OCP, organochlorine pesticide; OR, odds ratio; PAH, polycyclic aromatic hydrocarbon; PBDE, polybrominated diphenyl ether; PCB, polychlorinated biphenyl; PM, particulate matter; SO_2, sulfur dioxide.

References

1. Su FC, Goutman SA, Chernyak S, et al. Association of Environmental Toxins With Amyotrophic Lateral Sclerosis. *JAMA Neurol*. Jul 1 2016;73(7):803-11. doi:10.1001/jamaneurol.2016.0594
2. Goutman SA, Boss J, Patterson A, Mukherjee B, Batterman S, Feldman EL. High plasma concentrations of organic pollutants negatively impact survival in amyotrophic lateral sclerosis. *J Neurol Neurosurg Psychiatry*. Aug 2019;90(8):907-912. doi:10.1136/jnnp-2018-319785
3. Vinceti M, Violi F, Tzatzarakis M, et al. Pesticides, polychlorinated biphenyls and polycyclic aromatic hydrocarbons in cerebrospinal fluid of amyotrophic lateral sclerosis patients: a case-control study. *Environ Res*. May 2017;155:261-267. doi:10.1016/j.envres.2017.02.025
4. Wang MD, Little J, Gomes J, Cashman NR, Krewski D. Identification of risk factors associated with onset and progression of amyotrophic lateral sclerosis using systematic review and meta-analysis. *Neurotoxicology*. Jul 2017;61:101-130. doi:10.1016/j.neuro.2016.06.015
5. Filippini T, Tesauro M, Fiore M, et al. Environmental and Occupational Risk Factors of Amyotrophic Lateral Sclerosis: A Population-Based Case-Control Study. *Int J Environ Res Public Health*. Apr 22 2020;17(8)doi:10.3390/ijerph17082882
6. Dickerson AS, Hansen J, Gredal O, Weisskopf MG. Study of Occupational Chromium, Iron, and Nickel Exposure and Amyotrophic Lateral Sclerosis in Denmark. *Int J Environ Res Public Health*. Nov 2 2020;17(21)doi:10.3390/ijerph17218086
7. Peters S, Broberg K, Gallo V, et al. Blood Metal Levels and Amyotrophic Lateral Sclerosis Risk: A Prospective Cohort. *Ann Neurol*. Jan 2021;89(1):125-133. doi:10.1002/ana.25932
8. Figueroa-Romero C, Mikhail KA, Gennings C, et al. Early life metal dysregulation in amyotrophic lateral sclerosis. *Ann Clin Transl Neurol*. Jun 2020;7(6):872-882. doi:10.1002/acn3.51006
9. Goutman SA, Feldman EL. Voicing the Need for Amyotrophic Lateral Sclerosis Environmental Research. *JAMA neurology*. Mar 2 2020;doi:10.1001/jamaneurol.2020.0051
10. Julian TH, Glascow N, Barry ADF, et al. Physical exercise is a risk factor for amyotrophic lateral sclerosis: Convergent evidence from Mendelian randomisation, transcriptomics and risk genotypes. *EBioMedicine*. May 26 2021;68:103397. doi:10.1016/j.ebiom.2021.103397
11. Yang L, Lv X, Du H, Wu D, Wang M. Causal effects of serum metabolites on amyotrophic lateral sclerosis: A Mendelian randomization study. *Prog Neuropsychopharmacol Biol Psychiatry*. Mar 8 2020;97:109771. doi:10.1016/j.pnpbp.2019.109771
12. Dickerson AS, Hansen J, Gredal O, Weisskopf MG. Amyotrophic Lateral Sclerosis and Exposure to Diesel Exhaust in a Danish Cohort. *Am J Epidemiol*. Aug 1 2018;187(8):1613-1622. doi:10.1093/aje/kwy069
13. Filippini T, Fiore M, Tesauro M, et al. Clinical and Lifestyle Factors and Risk of Amyotrophic Lateral Sclerosis: A Population-Based Case-Control Study. *Int J Environ Res Public Health*. Jan 30 2020;17(3)doi:10.3390/ijerph17030857
14. Myung W, Lee H, Kim H. Short-term air pollution exposure and emergency department visits for amyotrophic lateral sclerosis: A time-stratified case-crossover analysis. *Environ Int*. Feb 2019;123:467-475. doi:10.1016/j.envint.2018.12.042

15. Peters S, Visser AE, D'Ovidio F, et al. Associations of Electric Shock and Extremely Low-Frequency Magnetic Field Exposure With the Risk of Amyotrophic Lateral Sclerosis. *Am J Epidemiol*. Apr 1 2019;188(4):796-805. doi:10.1093/aje/kwy287

16. Pupillo E, Poloni M, Bianchi E, et al. Trauma and amyotrophic lateral sclerosis: a european population-based case-control study from the EURALS consortium. *Amyotroph Lateral Scler Frontotemporal Degener*. Feb 2018;19(1-2):118-125. doi:10.1080/21678421.2017.1386687

17. Seals RM, Kioumourtzoglou MA, Gredal O, Hansen J, Weisskopf MG. Occupational formaldehyde and amyotrophic lateral sclerosis. *Eur J Epidemiol*. Oct 2017;32(10):893-899. doi:10.1007/s10654-017-0249-8

18. Seelen M, Toro Campos RA, Veldink JH, et al. Long-Term Air Pollution Exposure and Amyotrophic Lateral Sclerosis in Netherlands: A Population-based Case-control Study. *Environ Health Perspect*. Sep 27 2017;125(9):097023. doi:10.1289/EHP1115

19. Vinceti M, Filippini T, Violi F, et al. Pesticide exposure assessed through agricultural crop proximity and risk of amyotrophic lateral sclerosis. *Environ Health*. Aug 29 2017;16(1):91. doi:10.1186/s12940-017-0297-2

20. Vinceti M, Malagoli C, Fabbi S, et al. Magnetic fields exposure from high-voltage power lines and risk of amyotrophic lateral sclerosis in two Italian populations. *Amyotroph Lateral Scler Frontotemporal Degener*. Nov 2017;18(7-8):583-589. doi:10.1080/21678421.2017.1332078

21. Visser AE, D'Ovidio F, Peters S, et al. Multicentre, population-based, case-control study of particulates, combustion products and amyotrophic lateral sclerosis risk. *J Neurol Neurosurg Psychiatry*. Aug 2019;90(8):854-860. doi:10.1136/jnnp-2018-319779