Epidemiology

Long-term traffic-related air pollutant exposure and amyotrophic lateral sclerosis diagnosis in Denmark: A Bayesian hierarchical analysis

--Manuscript Draft--

Manuscript Number:	EDE21-0753R4
Full Title:	Long-term traffic-related air pollutant exposure and amyotrophic lateral sclerosis diagnosis in Denmark: A Bayesian hierarchical analysis
Article Type:	Original Article
Corresponding Author:	Robbie M Parks, Ph.D. Columbia University NY, New York UNITED STATES
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	Columbia University
Corresponding Author's Secondary Institution:	
First Author:	Robbie M Parks, Ph.D.
First Author Secondary Information:	
Order of Authors:	Robbie M Parks, Ph.D.
	Yanelli Nunez, PhD
	Arin A Balalian, MD, MPH
	Elizabeth A Gibson, PhD
	Johnni Hansen, PhD
	Ole Raaschou-Nielsen, PhD
	Matthias Ketzel, PhD
	Jibran Khan, PhD
	Jørgen Brandt, PhD
	Roel Vermeulen, PhD
	Susan Peters, PhD
	Jeff Goldsmith, PhD
	Diane B Re, PhD
	Marc G Weisskopf, PhD, ScD
	Marianthi-Anna Kioumourtzoglou, ScD
Order of Authors Secondary Information:	
Abstract:	Background: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease. Limited evidence suggests ALS diagnosis may be associated with air pollution exposure and specifically traffic-related pollutants. Methods: In this population-based case–control study, we used 3,937 ALS cases from the Danish National Patient Register diagnosed during 1989–2013 and matched on age, sex, year of birth, and vital status to 19,333 population-based controls free of ALS at index date. We used validated predictions of elemental carbon (EC), nitrogen oxides (NOx), carbon monoxide (CO), and fine particles (PM2.5) to assign 1-, 5-, and 10-year average exposures pre-ALS diagnosis at study participants' present and historical residential addresses. We used an adjusted Bayesian hierarchical conditional logistic model to estimate individual pollutant associations and joint and average associations for traffic-related pollutants (EC, NOx, CO).

	Results: For a standard deviation (SD) increase in 5-year average concentrations, EC (SD=0.42µg/m3) had a high probability of individual association with increased odds of ALS (11.5%; 95% credible interval [CrI] -1.0%, 25.6%; 96.3% posterior probability of positive association), with negative associations for NOx (SD=20µg/m3) (-4.6%; 95% CrI 18.1%, 8.9%; 27.8% posterior probability of positive association), CO (SD=106µg/m3) (-3.2%; 95% CrI 14.4%,10.0%; 26.7% posterior probability of positive association) and a null association for non-EC PM2.5 (SD=2.37µg/m3) (0.7%; 95%CrI 9.2%,12.4%). We found no association between ALS and joint or average traffic pollution concentrations. Conclusions: This study found high probability of a positive association between ALS diagnosis and EC concentration. Further work is needed to understand the role of traffic-related air pollution in ALS pathogenesis.
Additional Information:	
Question	**Response**
Word count, abstract (Enter "0" if not applicable.)	251
Word count, main text. Enter the number of words in the main text. For research articles, main text typically includes the introduction, methods, results, and discussion. Do not include words in title page, bibliography, tables, figure legends, or figures. Do not paste the text from the document itself into this box.	4154
Total word count. Enter the total word count for your submission, excluding the title page and abstract. Include the following in the total word count: (a) main text (for research articles, this typically includes the introduction, methods, results, and discussion), (b) bibliography, (c) tables, (d) figure legends, and (e) figures (calculated as 250 words per figure, each panel in a panel figure should be separately counted). Do not count words in Supplemental Digital Content. Do not paste the text from the document itself into this box.	6784
Funding sources All relevant sources of funding should be included here and on the title page of the manuscript with the heading “Source of Funding.” -Enter “none” if the work was completed without specific funding support. -If the result reported in the submission corresponds directly to the specific aims of a source (or sources) of funding, then describe that source of funding as: “The results reported herein correspond to specific aims of grant (or other source of support) XXX to investigator YYY from ZZZ”, where XXX is a grant or project	Robbie M Parks was supported by the NIEHS K99 ES033742 and the Earth Institute post-doctoral research fellowship at Columbia University. Funding was also provided by the National Institute of Environmental Health Sciences (NIEHS) grants R01 ES030616, R01 ES028805, R01 AG066793, R21 ES028472, P30 ES009089, and P30 ES000002.

number, YYY is the Principal Investigator of the grant or project, and ZZZ is the funding agency. -Describe all other sources of support as: "This work was (also) supported by grant(s) (or other source of support) XXX from ZZZ", where '(also)' is inserted only if the listed support is in addition to support corresponding directly to a specific aim, XXX is a grant or project number, and ZZZ is a funding agency. Additional sources of support should be added serially (e.g., grants XXX1 from ZZZ1, XXX2 from ZZZ2, and XXX3 from ZZZ3." Sources of support can include general salary support, which may not have a grant or project number. Grant or project numbers should be provided in a format that allows interested parties to find the grant in publicly available databases provided by many funding agencies.	
Describe the process by which someone else could obtain the data and computing code required to replicate the results reported in your submission. If one or the other is not available, please write "The [data, computing code, or both] are not available for replication because" and add an explanation for why one or the other is not available. Authors of submissions without original results, such as commentaries or letters, should enter "Not applicable." Your answer to this question will not affect the editors' decision about the submission's suitability for publication. If your paper is published, your response to this question will be included in the manuscript footnotes.	Danish patient records are available via the Danish National Patient Register (https://econ.au.dk/the-national-centre-for-register-based-research/danish-registers/the-national-patient-register/browse). Danish population records are available via the Danish Civil Registration System (https://econ.au.dk/the-national-centre-for-register-based-research/danish-registers/the-danish-civil-registration-system-cpr/browse). Exposure data are available via the DEHM-UBM-AirGIS website (https://envs.au.dk/en/research-areas/air-pollution-emissions-and-effects/the-monitoring-program/air-pollution-models/airgis/about-airgis/). All code for analysis, results from analysis, and visualization presented in this manuscript will be publicly available via GitHub at https://github.com/rmp15/multipollutants_and_als_code_review.
RETAINED RIGHTS: Except for copyright, other proprietary rights related to the Work (e.g., patent or other rights to any process or procedure) shall be retained by the author. To reproduce any text, figures, tables, or illustrations from this Work in future works of their own, the author must obtain written permission from Wolters Kluwer Health, Inc. ("WKH").	I agree

ORIGINALITY: Each author warrants that his or her submission to the Work is original, does not infringe upon, violate, or misappropriate any copyright or other intellectual property rights, or any other proprietary right, contract or other right or interest of any third party, and that he or she has full power to enter into this agreement. Neither this Work nor a similar work has been published nor shall be submitted for publication elsewhere while under consideration by this Publication.

AUTHORSHIP RESPONSIBILITY: Each author warrants that he or she has participated sufficiently in the intellectual content, the analysis of data, if applicable, and the writing of the Work to take public responsibility for it. Each has reviewed the final version of the Work, believes it represents valid work, and approves it for publication. Moreover, should the editors of the Publication request the data upon which the work is based, they shall produce it.

PREPRINTS: Upon acceptance of the article for publication, each author warrants that he/she will promptly remove any prior versions of this Work (normally a preprint) that may have been posted to an electronic server.

DISCLAIMER: Each author warrants that this Work contains no libelous or unlawful statements and does not infringe or violate the publicity or privacy rights of any third party, libel or slander any third party, contain any scandalous, obscene, or negligently prepared information, or infringe or violate any other personal or proprietary right of others. Each author

warrants that the Work does not contain any fraudulent, plagiarized or incorrectly attributed material. Each author warrants that all statements contained in the Work purporting to be facts are true, and any formula or instruction contained in the Work will not, if followed accurately, cause any injury, illness, or damage to the user. If excerpts (e.g., text, figures, tables, illustrations, or audio/video files) from copyrighted works are included, a written release will be secured by the author prior to submission, and credit to the original publication will be properly acknowledged. Each author further warrants that he or she has obtained, prior to submission, written releases from patients whose names or likenesses are submitted as part of the Work. Should the Editor or WKH request copies of such written releases, the author shall provide them in a timely manner.

TRADE COMPLIANCE: Each author warrants that if the author, any of the author's coauthors or any other individual whose content is included in the Work resides in Iran, Cuba, Syria, North Korea, Crimea, the Donetsk People's Republic (DNR) or the Luhansk People's Republic (LNR) regions of the Ukraine, the Work has been prepared in a personal, academic, or research capacity and not as an official representative or otherwise on behalf of the relevant government.

DISCLOSURES/CONFLICT OF INTEREST
Each author must identify any financial interests or affiliations with institutions, organizations, or companies relevant to the manuscript by completing the form below. Additionally, any financial associations involving a spouse, partner or children must be disclosed as well.

Note: Some sections below come from the ICMJE Uniform Disclosure Form for Potential Conflicts of Interest at http://www.icmje.org/downloads/coi_disclosure.pdf (dated July 2010).	
Did you or your institution at any time receive payment or support in kind for any aspect of the submitted work (including but not limited to grants, consulting fee or honorarium, support for travel to meetings for the study or other purposes, fees for participation in review activities such as data monitoring boards, statistical analysis, end point committees, and the like, payment for writing or reviewing the manuscript, provision of writing assistance, medicines, equipment, or administrative support, etc...)?	No
Other: Did you or your institution at any time receive additional payments or support in kind for any aspect of the submitted work?	No
Please indicate whether you have financial relationships (regardless of amount of compensation) with entities. You should report relationships that were present during the 36 months prior to submission including board membership, consultancy, employment, expert testimony, grants/grants pending, payment for lectures including service on speakers bureaus, payment for manuscript preparation, patents (planned, pending or issued), royalties, payment for development of educational presentations, stock/stock options, travel/accommodations/meeting expenses unrelated to activities listed (for example, if you report a consultancy above there is no need to report travel related to that consultancy), etc.	No
Other (err on the side of full disclosure): Please indicate whether you have any additional financial relationships (regardless of amount of compensation) with entities. You should report relationships that were present during the 36 months prior to submission.	None
Other Relationships	No other relationships/conditions/circumstances that present potential conflict of

Are there other relationships or activities that readers could perceive to have influenced, or that give the appearance of potentially influencing, what you wrote in the submitted work?	interest
AUTHOR'S OWN WORK: In consideration of WKH's publication of the Work, the author hereby transfers, assigns, and otherwise conveys all his/her copyright ownership worldwide, in all languages, and in all forms of media now or hereafter known, including electronic media such as CD-ROM, Internet, and Intranet, to WKH. If WKH should decide for any reason not to publish the Work, WKH shall give prompt notice of its decision to the corresponding author, this agreement shall terminate, and neither the author nor WKH shall be under any further liability or obligation. Each author grants WKH the rights to use his or her name and biographical data (including professional affiliation) in the Work and in its or the journal's promotion. Notwithstanding the foregoing, this paragraph shall not apply, and any transfer made pursuant to this paragraph shall be null and void if (i) the Work has been accepted by WKH for publication, and (ii) the author chooses to have the Work published by WKH as an open access publication. WORK MADE FOR HIRE: If this Work or any element thereof has been commissioned by another person or organization, or if it has been written as part of the duties of an employee, an authorized representative of the commissioning organization or employer must also sign this form stating his or her title in the organization. GOVERNMENT EMPLOYEES: If the Work or a portion of it has been created in the course of any author's employment by the United States Government, check the "Government" box at the end of this form. A work prepared by a	I agree

government employee as part of his or her official duties is called a "work of the U.S. Government" and is not subject to copyright. If it is not prepared as part of the employee's official duties, it may be subject to copyright. INSTITUTIONAL REVIEW BOARD/ANIMAL CARE COMMITTEE APPROVAL: Each author warrants that his or her institution has approved the protocol for any investigation involving humans or animals and that all experimentation was conducted in conformity with ethical and humane principles of research. WARRANTIES: Each author warranty made in this form is for the benefit of WKH and the Editor; each author agrees to defend, indemnify, and hold harmless those parties for any breach of such warranties.	
The journal will permit the author(s) to deposit for display a "final peer-reviewed manuscript" (the final manuscript after peer-review and acceptance for publication but prior to the publisher's copyediting, design, formatting, and other services) 12 months after publication of the final article on the author's personal web site, university's institutional repository or employer's intranet, subject to the following: * You may only deposit the final peer-reviewed manuscript. * You may not update the final peer-reviewed manuscript text or replace it with a proof or with the final published version. * You may not include the final peer-reviewed manuscript or any other version of the article on any commercial site or in	I agree

any repository owned or operated by any third party. For authors of articles based on research funded by the National Institutes of Health ("NIH"), Wellcome Trust, Howard Hughes Medical Institute ("HHMI"), or other funding agency, see below for the services that WKH will provide on your behalf to comply with "Public Access Policy" guidelines. * You may not display the final peer-reviewed manuscript until twelve months after publication of the final article. * You must attach the following notice to the final peer-reviewed manuscript: "This is a non-final version of an article published in final form in (provide complete journal citation)". * You shall provide a link in the final peer-reviewed manuscript to the journal website.	
"Public Access Policy" Funding Disclosure Please disclose below if you have received funding for research on which your article is based from any of the following organizations:	National Institutes of Health (NIH)
Please select:	Author's Own Work
Any additional comments?	None
Compliance with RCUK and Wellcome Trust Open Access Policies Both the Research Councils UK (RCUK) and the Wellcome Trust have adopted policies regarding Open Access to articles that have been funded by grants from the RCUK or the Wellcome Trust. If either “Wellcome Trust” or “Research Councils UK (RCUK)” has been selected above, and the authors of the applicable article choose to have the article published as an open access publication, the following policies will apply:	I agree

* If the article is to be published pursuant to the “Gold” route of Open Access, both the RCUK and the Wellcome Trust require that WKH make the article freely available immediately pursuant to the Attribution 4.0 Creative Commons License, currently found at http://creativecommons.org/licenses/by/4.0/legalcode (the “CC BY License”). The CC BY License is the most accommodating of the Creative Commons licenses and allows others to distribute, remix, tweak, and build upon the article, even commercially, as long as they credit the authors for the original creation.

* If the article is to be published pursuant to the “Green” route of Open Access, both the RCUK and the Wellcome Trust require that WKH make the article freely available within six months pursuant to the Attribution-NonCommerical 4.0 Creative Commons License, currently found at http://creativecommons.org/licenses/by-nc/4.0/legalcode (the “CC BY-NC License”). The CC BY-NC License allows others to remix, tweak, and build upon the article non-commercially, and although their new works must also acknowledge the authors for the original creation and be non-commercial, they don't have to license their derivative works on the same terms.

As a service to our authors, WKH will identify the National Library of Medicine (NLM) articles that require deposit pursuant to the RCUK and Wellcome Trust policies described in this section. This Copyright Transfer Agreement provides the mechanism for identifying such articles.

WKH will transmit the final peer-reviewed manuscript of an article based on research funded in whole or in part by either RCUK or the Wellcome Trust to Pub Med Central. Upon NIH request, it remains the legal responsibility of the author to confirm with NIH the provenance of his/her manuscript for purposes of deposit. Author will not deposit articles him/herself. Author will not alter the final peer-reviewed manuscript already transmitted to NIH. With respect to the “Green” route of Open Access, author will not authorize the display of the final peer-reviewed manuscript prior to 6 months following publication of the final article. Authors of articles that have been funded from grants from the RCUK or the Wellcome Trust are required to sign the WKH Open Access License Agreement prior to publication of the applicable article. Please contact the Editorial Office of the applicable journal to receive the Open Access License Agreement that is to be signed in connection with the publication of the article.	
I am the person in question for this submission or otherwise have approval to complete this agreement.	I agree
CME/CE Disclosure Each author must identify and disclose any financial associations involving a spouse, partner or children by completing the Family Disclosure question below, and whether any off-label uses or unapproved drugs or devices are discussed in his/her manuscript by completing the Off-Label Use/Unapproved Drugs or Products question below. In the event that the Work is published as a	I agree

continuing education or continuing medical education article, this information will be provided to the accrediting body and may be included in the published article. When applicable, articles accepted for publication may need to comply with additional standards related to CME or CE accreditation. Please refer to guidelines for authors for details. WKH and its affiliates reserve the right to publish the manuscript as a continuing education article.	
Family Disclosure Do your children or your spouse or partner have financial relationships with entities that have an interest in the content of the submitted work?	No other relationships/conditions/circumstances that present potential conflict of interest
Off-Label Use/Unapproved Drugs or Products If your manuscript discusses an unlabeled use of a commercial product or device or an investigational use of a product or device not yet approved by the FDA for any purpose, you must specifically disclose in the manuscript that the product is not labeled for the use under discussion or that the product is still investigational. Please check the item below that applies to you	I will not discuss unlabeled/investigational uses of any commercial product or device

THE EARTH INSTITUTE
COLUMBIA UNIVERSITY

The Earth Institute
Columbia University
Hogan Hall
MC 3277
2910 Broadway
NY, NY 10025

1 June 2022

Robbie M Parks
Post-doctoral research fellow
Earth Institute, Columbia University
robbie.parks@columbia.edu
www.robbiemparks.github.io

Dr Timothy L. Lash,
Editor-in-Chief, *Epidemiology*

Dr Stefanie Ebelt
Editor, *Epidemiology*

Dear Dr Lash, Dr Ebelt:

Please find enclosed our revised manuscript entitled “Long-term traffic-related air pollutant exposure and amyotrophic lateral sclerosis diagnosis in Denmark: A Bayesian hierarchical analysis” (DE21-0753). We have revised the text according to the comments and the suggestions of the Editors and Reviewers, as outlined in our responses.

We look forward to your response and would be glad to address any further comments you may have on the manuscript.

Amyotrophic lateral sclerosis (ALS) is a devastating and fatal neurodegenerative disease, currently without a cure. Approximately half of patients die within three years of symptom onset. Annually, there are nearly 30,000 cases of ALS in Europe and over 200,000 worldwide. Known inherited genetic variants only account for 5–10% of ALS cases. Environmental factors, therefore, are likely important in ALS pathogenesis. Ambient air pollution, especially urban air pollution, is a ubiquitous exposure that has been associated with several other neurodegenerative disorders, and is consistently linked to systemic inflammation, oxidative stress, and neuroinflammation, all of which, in turn, have been reported as key pathways to ALS pathogenesis. Despite the compelling plausibility, few studies to date have evaluated the association between air pollution and ALS.

With this study, *the largest case-control study of ALS and air pollution to date,* we used data on 3,939 ALS cases from the Danish National Patient Register diagnosed between 1989 – 2013 and matched on, sex, year of birth and vital status to 19,298 population-based controls free of ALS at index date. We used predictions of nitrogen oxides (NO_x), carbon monoxide (CO), elemental carbon (EC), and fine particles ($PM_{2.5}$) from validated spatio-temporal models to assign 1-, 5-, and 10-year average exposures pre-ALS diagnosis at present and historical residential addresses of study participants. Although the last year of data in our analyses is from eight years ago (2013), our findings remain timely due to the uniquely large number of ALS cases we have collected, that air pollution remains so pervasive, and that ALS prevalence is projected to increase nearly 70% by 2040.

We found that an increase in the joint exposure to traffic-related pollutants was associated with an increase in odds of ALS diagnosis, significant for elemental carbon for a 1-year average standard deviation increase, though not significant at the 95% credible interval level for other pollutants. Our results indicate that sources of air pollution with elemental carbon, such as diesel engines and woodburning stoves, might contribute to development of ALS.

This manuscript has not been previously published and is not under review in any other journal. All authors have contributed to the paper, have approved its submission, and take responsibility for its contents. The authors have no actual or potential competing financial interests. There is no closely related paper included with this submission.

Sincerely,

Robbie M. Parks, PhD

MS# EDE21-0753

Type of Manuscript: Original Research Article

Manuscript Title: Long-term traffic-related air pollutant exposure and amyotrophic lateral sclerosis diagnosis in Denmark: A Bayesian hierarchical analysis

Authors: Robbie M Parks[1,2*], Yanelli Nunez[1], Arin A Balalian[3], Elizabeth A Gibson[1,4], Johnni Hansen[5], Ole Raaschou-Nielsen[5,6], Matthias Ketzel[6,7], Jibran Khan[6], Jørgen Brandt[6,8], Roel Vermeulen[9], Susan Peters[9], Jeff Goldsmith[10], Diane B. Re[1], Marc G. Weisskopf[11], Marianthi-Anna Kioumourtzoglou[1]

[1]Department of Environmental Health Sciences, Mailman School of Public Health, Columbia University, New York, New York, USA
[2]The Earth Institute, Columbia University, New York, New York, USA
[3]Department of Epidemiology, Mailman School of Public Health, Columbia University, New York, New York, USA
[4]Department of Biostatistics, Harvard TH Chan School of Public Health, Boston, Massachusetts, USA
[5]Danish Cancer Society Research Center, Copenhagen, Denmark
[6]Department of Environmental Science, Aarhus University, Roskilde, Denmark
[7]Global Centre for Clean Air Research (GCARE), University of Surrey, Guildford, United Kingdom
[8]iClimate – interdisciplinary Center for Climate Change, Aarhus University, Denmark
[9]Institute for Risk Assessment Sciences, Utrecht University, Utrecht, the Netherlands
[10]Department of Biostatistics, Mailman School of Public Health, Columbia University, New York, New York, USA
[11]Departments of Environmental Health and Epidemiology, T. H. Chan School of Public Health, Harvard University, Boston, Massachusetts, USA

[*]**Corresponding Author:**
Robbie M Parks
Department of Environmental Health Sciences
Columbia University Mailman School of Public Health
722 West 168th Street, #1104
New York, New York, 10032
Email: robbie.parks@columbia.edu

Suggestions for running head: Traffic-related air pollutants and ALS

The authors declare they have no actual or potential competing financial interests.

Sources of financial support: Robbie M Parks was supported by the NIEHS K99 ES033742 and the Earth Institute post-doctoral research fellowship at Columbia University. Funding was also provided by the National Institute of Environmental Health Sciences (NIEHS) grants R01

ES030616, R01 ES028805, R01 AG066793, R21 ES028472, P30 ES009089, and P30 ES000002.

Description of the process by which someone else could obtain the data and computing code required to replicate the results reported in your submission (or explanation why data or code are not available): Danish patient records are available via the Danish National Patient Register (https://econ.au.dk/the-national-centre-for-register-based-research/danish-registers/the-national-patient-register/browse). Danish population records are available via the Danish Civil Registration System (https://econ.au.dk/the-national-centre-for-register-based-research/danish-registers/the-danish-civil-registration-system-cpr/browse). Exposure data are available via the DEHM-UBM-AirGIS website (https://envs.au.dk/en/research-areas/air-pollution-emissions-and-effects/the-monitoring-program/air-pollution-models/airgis/about-airgis/). All code for analysis, results from analysis, and visualization presented in this manuscript will be publicly available via GitHub at https://github.com/rmp15/multipollutants_and_als_code_review.

Abstract

Background: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease. Limited evidence suggests ALS diagnosis may be associated with air pollution exposure and specifically traffic-related pollutants.

Methods: In this population-based case–control study, we used 3,937 ALS cases from the Danish National Patient Register diagnosed during 1989–2013 and matched on age, sex, year of birth, and vital status to 19,333 population-based controls free of ALS at index date. We used validated predictions of elemental carbon (EC), nitrogen oxides (NO_x), carbon monoxide (CO), and fine particles ($PM_{2.5}$) to assign 1-, 5-, and 10-year average exposures pre-ALS diagnosis at study participants' present and historical residential addresses. We used an adjusted Bayesian hierarchical conditional logistic model to estimate individual pollutant associations and joint and average associations for traffic-related pollutants (EC, NO_x, CO).

Results: For a standard deviation (SD) increase in 5-year average concentrations, EC (SD=0.42μg/m^3) had a high probability of individual association with increased odds of ALS (11.5%; 95% credible interval [CrI] -1.0%, 25.6%; 96.3% posterior probability of positive association), with negative associations for NO_x (SD=20μg/m^3) (-4.6%; 95% CrI 18.1%, 8.9%; 27.8% posterior probability of positive association), CO (SD=106μg/m^3) (-3.2%; 95% CrI 14.4%,10.0%; 26.7% posterior probability of positive association) and a null association for non-EC $PM_{2.5}$ (SD=2.37μg/m^3) (0.7%; 95%CrI 9.2%,12.4%). We found no association between ALS and joint or average traffic pollution concentrations.

Conclusions: This study found high probability of a positive association between ALS diagnosis and EC concentration. Further work is needed to understand the role of traffic-related air pollution in ALS pathogenesis.

Abbreviations:

ALS	Amyotrophic lateral sclerosis
BKMR	Bayesian kernel machine regression
BMI	Body mass index
CO	Carbon monoxide
CrI	Credible interval
DEHM-UBM-AirGIS	Spatio-temporal air pollution modeling system used in study
EC	Elemental carbon
ICD	International Classification of Diseases
IQR	Interquartile range
IR	Incidence ratio
Non-EC $PM_{2.5}$	Non-elemental carbon fine particles
NO_x	Nitrogen oxides
O_3	Ozone
$PM_{2.5}$	Fine particles
SD	Standard deviation
SES	Socioeconomic status

Introduction

Amyotrophic lateral sclerosis (ALS) is a devastating and fatal neurodegenerative disease,[1] currently without a cure.[2] Approximately half of patients die within 3 years of symptom onset.[3] Annually, there are nearly 30,000 cases of ALS in Europe and over 200,000 worldwide.[4] Known inherited genetic variants only account for 5–10% of ALS cases.[5,6] Environmental factors, therefore, are likely important in ALS pathogenesis.[7] However, because the disease is relatively rare, it is challenging to conduct large-scale prospective studies. There is a recognized need for more evidence of the environmental contributors of ALS.[5,8]

Although air pollution is commonly studied in association with respiratory- and cardiovascular-related outcomes, e.g., references [9–14], epidemiologic and toxicologic studies also support several plausible biologic mechanisms in association with the nervous system and neurodegeneration, e.g., references [15–34]. Ambient air pollution, especially urban air pollution, is a ubiquitous exposure that has been associated with several other neurodegenerative disorders, e.g., references [16–21,35,36]. and is consistently linked to systemic inflammation,[22–24] oxidative stress,[25–28] and neuroinflammation,[15,29] all of which, in turn, have been reported as key pathways to ALS pathogenesis, e.g., references [30–34].

Despite the compelling plausibility, few studies to date have evaluated the association between air pollution and ALS.[35,37–39] A study in 2021 found that traffic-related air pollutants may be driving observed associations.[38] Another study of ALS and $PM_{2.5}$ in Denmark examining critical windows of exposure found that more recent exposure to $PM_{2.5}$ (i.e., the previous 1 to 5 years) may be the most important driver of the potential association, though the constituents of $PM_{2.5}$

were not analyzed, neither together nor separately.[40] To our knowledge no study has hitherto attempted to understand the individual, joint, and average associations of the pollutants in a single model. Air pollutants have been consistently associated with adverse health, primarily in single pollutant analyses.[13,17,41–43] However, they are highly correlated with one another.[41] It is therefore a mixture modeling challenge to infer the association of multiple air pollutants and health outcomes.[44] Using three air pollutants commonly used in health studies as traffic-related emissions tracers— elemental carbon (EC), nitrogen oxides (NO_x), and carbon monoxide (CO)— we aimed to assess whether exposure to (a) each individual air pollutant is independently associated with ALS diagnosis, and estimate their (b) joint and (c) average traffic-related emissions associations. This study pairs with and complements the work of Nunez et al.[40]

Methods

Study Population and Outcome Assessment

We used data from the Danish National Patient Register during 1989-2013, through which details on demographic characteristics and certain health outcomes of all Danish residents can be linked based on a unique personal identifier.[45] The Register was established in 1977 and is comprehensive, including nationwide clinical and administrative records for all inpatient data, with outpatient data available since 1995.[46]

We identified ALS cases based on their International Classification of Diseases (ICD) discharge diagnoses, i.e., ICD-8 code 348.0 (ALS) until 1993 and ICD-10 code G12.2 (motor neuron disease) thereafter, using the date of the first relevant code as the diagnosis date. This was the index date. We only included patients who were at least 20 years old when diagnosed because (i)

cases younger than 20 years old were at a greater chance of misclassification, since ALS has been predominantly diagnosed in older adults,[47] and (ii) the very few juvenile ALS cases have been explained to a much larger degree by genetic mutations (~40%).[48] In our validation study, Register data for ALS ascertainment were highly reliable; working with a specialist ALS neurologist to review medical records and comparing to death certificates and hospital discharges, the Danish National Patient Register was found to have an overall predictive value for ALS of 82%.[49]

We obtained controls through the Danish Civil Registration System, established in 1968 and updated daily, which includes administrative records (e.g., date and place of birth, sex, vital status, and history of civil status and addresses since 1971) on all persons living in Denmark; records are kept even when a person dies or emigrates.[50] We randomly matched five controls per case by age, sex, year of birth, and vital status. Controls were alive and free of diagnosed ALS at the ALS diagnosis date of the matched case (index date). The control-sampling scheme followed a risk-set matching pattern, so cases could have served as controls before diagnosis of ALS.[51]

We obtained all addresses of cases and controls from 1 January 1979 onwards from the Danish Civil Registration System,[50] including the dates of moving to and from each address, before the index date. We then obtained the geographical coordinates at the door of each house of the residential history of the participants, with previous evidence of the high accuracy of this method of geocoding of addresses in Denmark.[17]

This study was approved by the Institutional Review Board Committee at Columbia University and the Danish Data Protection Agency.

Exposure data

We obtained predictions on monthly concentrations of elemental carbon (EC), nitrogen oxides (NO_x), carbon monoxide (CO), and fine particles ($PM_{2.5}$) (as well as ozone, O_3, for a sensitivity analysis, usually negatively correlated with other pollutants due to its chemistry[52]), at residential addresses of study participants from the validated spatio-temporal air pollution modeling system DEHM-UBM-AirGIS that provides full space and time coverage over the study period, described in detail elsewhere.[53–56] In brief, DEHM-UBM-AirGIS is a human exposure modeling system for traffic pollution, developed for application in Danish air pollution epidemiologic studies. The modeling system integrates air pollution dispersion models, digital maps, national and local administrative databases, concentrations of air pollutants at regional, urban background and street level, meteorologic data, and a Geographic Information System (GIS). The modeling system is therefore able to generate street configuration and traffic data based on digital maps and national databases, which enables estimation of air quality levels at a large number of addresses in an automatic and effective way. These predicted pollutant concentrations have been extensively used in previous air pollution epidemiologic studies in Denmark.[17,40,57–59] The models have good predictive accuracy, with average monthly correlations between measured and modeled results of 0.79 for EC, 0.85 for NO_x, 0.91 for CO, 0.92 for O_3, and 0.83 for annual concentrations of $PM_{2.5}$.[53,56] Because traffic is a major source of $PM_{2.5}$ and EC one of the main $PM_{2.5}$ components in urban environments,[60] we removed the EC concentration from the total $PM_{2.5}$ mass concentration (non-EC $PM_{2.5}$) by subtraction to avoid overadjustment when

including both in the models simultaneously; this was valid since the DEHM-UBM-AirGIS modeling system constructed $PM_{2.5}$ concentrations by adding from specific species of pollutants, one of which was EC.[53–56]

Based on the residential history of each case or control, we calculated 1-, 5-, and 10-year average exposure to each pollutant ending at 1 year before the index date, as diagnosis has been shown previously to occur at a median of 12 months after symptoms onset.[61] Specifically, each case or control average value (1-, 5- or 10-year) was calculated as the mean of all concentrations recorded across time at the recorded addresses within each time window. A small number of Danish residents lack a complete address history (1.7%; lack of house number). To ensure we were including participants with adequately complete exposure records, we set the following minimum criteria for number of complete exposure record months to include cases and controls: (i) 1-year averages: 9 of 12 months, at least one measurement in each season; (ii) 5-year averages (main exposure): 30 of 60 months; and (iii) 10-year averages: 60 of 120 months.

Covariate data

We included a set of covariates based on as close as possible to index date to account for potential confounding bias, including household socioeconomic status (SES) based on last-reported job title at index date; civil status at index date, last reported place of residence at index date, and place of birth. We used a five-category, individual-level SES definition developed by the Danish Institute of Social Sciences, based on job titles from income tax forms, which has been associated with ALS diagnosis in Denmark,[62] as well as how quickly one is identified as having ALS in the Danish Civil Registration System.[63] Group 1 (highest status) includes

corporate managers and academics; group 2: proprietors, managers of small businesses and teachers; group 3: technicians and nurses; group 4: skilled workers; and group 5: unspecialized workers, such as entry-level positions within food and retail environments. We also included a group for participants whose job title was unknown (group 9). For each married participant, we used the higher of the couple's individual SES categories, when available. We also used information on civil status (never married, married, divorced, widowed) due to the influence that a spouse may have on visiting a family physician,[64] last reported place of residence from postcode (Greater Copenhagen, big cities of Denmark, rest of Denmark, Greenland) to account for various local environmental and behavioral stressors,[7] and place of birth (Greater Copenhagen, big cities of Denmark, rest of Denmark, Greenland, foreign, unknown) to adjust for other potential family-specific, location-specific, and early-life confounders, which may have an impact on the probability of developing ALS.[65] Ultimately, we were limited by what was available in the Danish Civil Registration System.[63] As part of a sensitivity analysis, we also included parish-level SES, measured by percentage of residents with greater than high-school education, in the model. In Denmark, parishes are administrative units with an average population of ~2,500 residents.

Statistical analysis

We analyzed the association between ALS diagnosis (binary) and exposure to traffic-related pollutants by applying a Bayesian formulation of the conditional logistic model, with Bayesian hierarchy on the traffic-related pollutants (EC, NO_x, CO).[66,67] The conditional approach examines contrasts within matched strata, i.e., groupings of case and matched controls, implicitly adjusting for matching factors (age, sex, year of birth, vital status) within each matched

stratum.[66] Matching by finer scale than year of birth was not possible. Bayesian inference allows for full distributional estimation of parameters of interest.[67] We employed a Bayesian hierarchical formulation because it enables estimates of (a) independent pollutant–outcome associations, (b) a joint association of the three pollutants (i.e., total percentage change in odds of ALS diagnosis with a simultaneous increase in each of EC, NO_x, CO), and (c) an average traffic association (i.e., average percentage change in odds of ALS diagnosis with increases in each of EC, NO_x, CO), while accounting for the variance–covariance structure between the highly correlated exposures and their coefficients.[67] We included a linear term for each included pollutant and adjusted for individual- and parish-level SES, civil status, last reported place of residence, and place of birth.

Specifically, via a logit function, we modeled the log-odds of ALS diagnosis, as follows:

$$logit[\Pr(\mathrm{Y}_{ci} = 1)] = \alpha_c +$$

$$\beta_{EC}\mathrm{EC}_{ci} + \beta_{\mathrm{NO}_x}\mathrm{NO}_{x\,ci} + \beta_{CO}\mathrm{CO}_{ci} +$$

$$\beta_{\mathrm{PM}_{2.5}}(\text{non-EC PM}_{2.5\,ci}) +$$

$$\beta_{\mathrm{SES}}\mathrm{SES}_{ci} + \beta_{\text{Civil Status}}\text{Civil Status}_{ci} + \beta_{\mathrm{Residence}}\mathrm{Residence}_{ci} + \beta_{\mathrm{Birth}}\mathrm{Birth}_{ci},$$

where Y_{ci} denotes whether subject i in matched stratum c was diagnosed with ALS, i.e., c represents a case and its matched controls; α_c the matched stratum-specific intercepts (not estimated in conditional logistic models); $\beta_{EC}, \beta_{\mathrm{NO}_x}, \beta_{CO}, \beta_{\mathrm{PM}_{2.5}}$ the individual pollutant coefficients (log-odds) per standard deviation (SD) increase in concentration of EC, NO_x, CO, non-EC $PM_{2.5}$ respectively, scaled by their respective SDs and centered at their means, with each β an individual pollutant association adjusted by other terms in the model and the rest as coefficients for subject-specific covariates. Interquartile range (IQR) could equivalently be used

to scale pollutant concentrations. If other sources of air pollution are associated with ALS, then including non-EC $PM_{2.5}$ adjusts for $PM_{2.5}$ from other sources,[68] as well as indicating whether pollution from other sources not explicitly quantified might also have associations with ALS. Therefore, $\beta_{\mathrm{PM}_{2.5}}$ is interpreted as the association with air pollutants not specifically included in our analysis. In urban European environments, traffic-related pollutants typically represent on-average 14% of $PM_{2.5}$ concentrations.[69] In a sensitivity analysis, we included O_3 in the model, as O_3 concentrations have been associated with many adverse health outcomes,[70] and were negatively correlated with traffic-related pollutants, and added $ns(\mathrm{SES}_{\mathrm{parish}_{ci}})$, as a natural spline with three degrees of freedom.

In our model, β_{EC}, β_{NO_x}, and β_{CO} represent the independent individual pollutant associations with ALS diagnosis. In the same model, we estimated the joint association between these three pollutants and ALS diagnosis as:

$$Traffic_{Joint} = \sum_{p=EC,NO_x,CO} \beta_p p.$$

This sum quantifies the association (log-odds) with ALS of a one-SD increase in the three pollutants simultaneously.

Finally, we assumed that the traffic-related individual pollutant associations arise from a distribution of the average traffic association with ALS diagnosis. We placed a hierarchy on the traffic-specific individual pollutant terms in the model to account for the fact that the traffic-related pollutants, EC, NO_x, CO, originate from common sources and primarily traffic in urban environments:

$$\beta_{Traffic} = [\beta_{EC}, \beta_{NO_x}, \beta_{CO}],$$

$$\beta_{Traffic} \sim MVN(\mu, \Sigma),$$

$$\mu \sim N(\lambda, \sigma_\lambda),$$

$$\Sigma = \tau\Omega\tau,$$

where λ denotes the average one-SD association of traffic-related pollution with variance σ_λ. Σ, the estimated variance–covariance matrix among individual pollutant estimates, was expressed as a decomposition into a positive-definite correlation matrix Ω and scale matrix τ.[71]

We used weakly informative priors so that data drove parameter estimation. Hyper-priors for coefficients on non-EC $PM_{2.5}$ and covariates were N(0,10); for σ_λ and τ we used Half-Cauchy(0,10), as recommended by Gelman, Polson, and Scott as a weakly-informative prior;[72,73] Ω was defined by the weakly-informative prior LKJCorr(1).[74] The exception to this was the prior for λ, the average association of traffic-related pollutants, for which estimates became unrealistically high (approaching infinity and not converging with further iterations) with a more weakly-informative prior. We therefore used a prior of N(0,0.1), which did not affect estimates of other parameters. We conducted sensitivity analyses to understand the influence of priors and the robustness of the results.

We present all results as percentage change in odds of ALS diagnosis per SD increase in pollutant concentration (calculated via e.g., $e^{\beta_{\mathrm{EC}}} - 1$, etc. obtained in the modeling process). Due to the risk-set matching pattern of our case–control study, odds ratios are also equivalently incidence ratios (IRs).[66] We ran each model with four chains with a sample size of 1,000 each, after a warm-up of 1,000 samples, for 4,000 total samples. We assessed whether the models

converged by checking that the Gelman–Rubin potential scale reduction statistic[75] was below 1.1 for all estimated model parameters. The reported 95% credible intervals (CrI) are the 2.5th to 97.5th percentiles of each parameter's posterior marginal distribution. To calculate the probability that an association estimate was greater than null, we used the 4,000 samples of the posterior distribution and took the proportion of samples which were above the null. A 50% probability means that it is as likely as not that the marginal estimate is null, a probability closer to 100% indicates that the association is more likely to be truly positive, with closer to 0% indicating more likely to be truly negative.

We conducted statistical analyses using the R Statistical Software, version 4.1.1[76] and R-STAN, version 2.21.2.[67] All code for analysis, results from analysis, and visualization presented in this manuscript is publicly available via GitHub at https://github.com/rmp15/traffic_air_pollution_als_denmark_epidemiology.

We assessed the sensitivity of our results to hyper-prior assignment; running more iterations and warm-up per chain; inclusion of O_3; single traffic-related pollutant models adjusting for non-EC $PM_{2.5}$; as well as adjusting by parish-level SES. From the parish-level SES sensitivity analysis we excluded those who lived in areas without parish-level SES data, namely: (i) 819 participants for 1-year average exposure; (ii) 826 participants for 5-year average exposure; and (iii) 838 participants for 10-year average exposure.

Results

After filtering the original 4,011 cases and 20,055 controls based on completeness of exposure records, we used information on 3,934 (98.1% of total) cases and 19,298 (96.2% of total) controls for 5-year average exposure. We also used 3,937 cases,19,333 controls for 1-year average exposure and 3,929 cases, 19,250 controls for 10-year average exposure. Descriptive statistics of included cases and controls for 5-year average exposure can be found in Table 1. Descriptive statistics of controls for 5-year exposure by socioeconomic status, civil status, residence, and place of birth are found in eTables 1-4. For the main results, we present 5-year average exposure associations as a balance between representation of most recent exposure as well as long-term concentration.

The 5-year average traffic-related pollutant concentrations were 0.85 $\mu g/m^3$ for EC (SD=0.42 $\mu g/m^3$), 27 $\mu g/m^3$ for NO_x (SD=20 $\mu g/m^3$), and 238 $\mu g/m^3$ for CO (SD=106 $\mu g/m^3$) (Table 2). Figure 1 shows Spearman correlations between pollutants for 1-, 5-, and 10-year average exposures. Traffic-related pollutants (EC, NO_x, CO) were highly correlated in cases, controls and overall, ranging from correlations of 0.91 to 0.96. Otherwise, non-EC $PM_{2.5}$ was most highly correlated with CO, ranging from 0.67 to 0.7. O_3 was negatively correlated with other pollutants, ranging from -0.54 to -0.89. Maps of average concentration of included pollutants (EC, NO_x, CO, $PM_{2.5}$, O_3) across Denmark for a representative year (2000; middle of study period 1989-2013) are also available in eFigure 1.

For 5-year average pollutant concentrations, we observed the largest overall increase in odds of ALS diagnosis for the individual SD increase in EC (11.5%; 95% CrI: -1.0%, 25.6% per 0.42 $\mu g/m^3$; 96.3% posterior probability of positive association) (Figure 2). SD increases were

associated with a decrease in odds of ALS diagnosis in NO_x (-4.6%; 95% CrI: -18.1%, 8.9% per 20 $\mu g/m^3$; 27.8% posterior probability of positive association) and CO (-3.2%; 95% CrI: -14.4%, 10.0% per 106 $\mu g/m^3$; 26.7% posterior probability of positive association). Non-EC $PM_{2.5}$ was not associated with ALS diagnosis (0.7%; 95% CrI: -9.2%, 12.4% per 2.37 $\mu g/m^3$; 54.1% posterior probability of positive association). One-year EC average exposure was associated with an increase in odds of ALS diagnosis (15.4%; 95% CrI: 1.6%, 25.6% per 0.42 $\mu g/m^3$; 98.9% posterior probability of positive association). Single-pollutant models for each traffic-related pollutant adjusting for non-EC $PM_{2.5}$ (eFigure 1; single traffic-related pollutant models D, E and F) resulted in positive associations for each of EC, NO_x, CO, with positive associations for non-EC $PM_{2.5}$ in all but the model with EC. The 95% credible interval for EC in the single-pollutant model (eFigure 1; model F) overlapped with the credible intervals of the EC term in the multi-pollutant models (eFigure 1; models A to C, G to P). The joint association of traffic-related pollutants (EC, NO_x, CO) was 2.3% (95% CrI: -3.3%, 7.7%), with an 77.8% posterior probability of a positive association. The average traffic association was null (-0.1%; 95% CrI: -17.4%, 20.8%; 45.5% posterior probability of positive association). Compared to the 1- and 5-year results, the 10-year average exposure results were attenuated, as associations tended further to the null. Results from variations of the main model in the sensitivity analyses were robust to prior choices, inclusion of O_3, and inclusion of parish-level SES (eFigure 2).

Discussion

In the largest case–control study of ALS and traffic-related air pollution to date (to our knowledge), we found that EC had the largest-in-magnitude independent association with ALS diagnosis, while associations with NO_x and CO were negative with credible intervals

overlapping the null, and smaller in magnitude. A joint increase in concentrations of traffic-related pollutants had a high probability of being associated with an increase in odds of ALS diagnosis. Sensitivity analyses demonstrated that for single pollutant models, the association for EC was smaller than for our main multi-pollutant model, which took into account the variance–covariance structure of traffic-related pollutants. Overall conclusions for the association between EC and ALS diagnosis were similar from the single- or multi-pollutant models. The inconsistent associations for NO_x and CO in the multi- and single-pollutant models and the consistency of the EC association suggest that EC concentrations may have been more relevant than NO_x and CO for ALS diagnosis, though further study is required. Our results indicate that traffic-related pollutants, hazardous in many ways,[9–21,41–43] may also be associated with ALS diagnosis. Our finding—that increases in EC are potentially positively associated with ALS diagnosis—is plausible. A case–control study in the Netherlands from 2021 reported that ultrafine particles, another traffic emissions-related surrogate, were associated with ALS diagnosis,[38] while another based in Catalonia, Spain found ALS cases clustered around key road infrastructure.[77] Although we did not find an association with non-EC $PM_{2.5}$ in our study, our results are not directly comparable to those of the other studies, as our $PM_{2.5}$ effect estimates capture the $PM_{2.5}$ components not accounted for by other pollutants in the analysis. A study examining critical windows of exposure of $PM_{2.5}$ and ALS diagnosis in Denmark found that concentrations 1 to 5 years before exposure may be driving the association with ALS onset,[40] consistent with our findings that the most recent 1-year average EC concentration exhibited the largest association.

Our results indicate that EC exposure—a large part of which comes from diesel combustion and small combustion sources (such as wood stoves) in European urban centers, where prevalence of

diesel cars is high[78]—has a high probability of a positive association with ALS diagnosis. In our previous study of ALS and occupational exposures in Denmark we found that those working in agriculture and construction, associated with exposure to diesel engine exhausts, were at higher relative risk than those in other employments.[62] Truck drivers, for whom diesel exposure is common, are also at increased risk of sporadic ALS.[79] EC exposure has been associated with inflammation,[80] mitochondrial dysfunction[81] and DNA damage,[81,82] all of which are plausible pathways of neurodegeneration. These factors have also previously been identified as particular pathways to pathogenesis of ALS.[30–34]

We did not find a high probability of a positive association with NO_x in our analyses, in contrast with a previous study, though that study did not include EC.[38] NO_x is also highly correlated with EC (0.94 to 0.96 in our study), which is expected given that they are both combustion products commonly associated with emissions in urban environments. EC exposure was more strongly associated with 1-year than for 5-/10-year average concentrations, which may indicate that the previous year of exposure may be the most relevant exposure window relevant to traffic-related exposures and ALS; this is biologically plausible, as this critical exposure window would be at the pre-symptomatic stage of underlying ALS progression, where traffic-related pollution exposure may add to the ongoing cellular or molecular process of the disease, to the point where the body can no longer compensate and subsequently enters the clinical phase.[83–85] We do not expect that these results are attributed to reverse causation, as we have lagged these 1-year exposures by one year already prior to diagnosis, and there was likely little substantial residential movement in the year before ALS diagnosis.[86] We do not expect that calendar time was a potential source of confounding, as the controls were matched on age and year of birth. The null

joint association, combined with the largest associations from traffic-related pollutant in all models found with EC, further indicates that EC may be driving the association of air pollution with ALS, though further analysis will be necessary to confirm this.

Our study had one of the largest numbers of ALS patients ever included in an environmental health study. Another strength of our study is that we leveraged highly correlated traffic pollutants and Bayesian hierarchical modeling and were able to estimate independent, joint, and average traffic-related pollutant associations. Although we have adjusted implicitly (by matching; age, sex, year of birth, vital status) and explicitly for many common covariates (SES, civil status, residence, place of birth), we cannot rule out residual confounding. Information on individual-level variables, such as body mass index (BMI) and smoking status is not currently available through the Danish Civil Registration System. These variables, while potential risk factors for ALS, are not likely confounders in this analysis as they are not expected to be associated with pollutant concentrations in a manner independent of neighborhood SES. If this information were available, it could be used to further adjust for SES.[86] To the extent that the variables we included in our models to adjust for household- and neighborhood-level SES are adequate, we would expect any residual SES-related confounding to be minimal. Exposure measurement error is inevitable, as any modeled exposure will be inaccurate to some degree. However, any error is not likely correlated with ALS diagnosis, and therefore any bias would be towards the null.[87] While a previous study found that ALS ascertainment from the Danish National Patient Register was highly reliable,[49] outcome misclassification cannot be ruled out, nor can the possibility that date of diagnosis and symptom onset were irregularly aligned. While our analysis adjusted for marital status and household SES, many couples in Denmark

cohabitate. This would not be captured by our analysis, and ALS diagnosis in relation to cohabitation status should be further investigated.[88]

Future research might use larger cohort data to understand the importance of each respective pollutant in a single model. Other mixture model methods, such as Bayesian Kernel Machine Regression[89] might be useful in further exploring the robustness of joint associations in a different framework, though this method was not appropriate for our particular research question, since it is currently not available for case–control study applications. The timing of exposure will continue to be an important study route. ALS is projected to increase in prevalence over the next few decades all over the world.[4] Understanding ALS pathogenesis and identifying modifiable risk factors is critical for preventive action.

References

1. Rowland LP, Shneider NA. Amyotrophic lateral sclerosis. *New England Journal of Medicine*. 2001;344(22):1688-1700.

2. Chio A, Logroscino G, Hardiman O, et al. Prognostic factors in ALS: A critical review. *Amyotrophic Lateral Sclerosis*. 2009;10(5-6):310-323.

3. Mitchell JD, Borasio GD. Amyotrophic lateral sclerosis. *The Lancet*. 2007;369(9578):2031-2041.

4. Arthur KC, Calvo A, Price TR, Geiger JT, Chio A, Traynor BJ. Projected increase in amyotrophic lateral sclerosis from 2015 to 2040. *Nature Communications*. 2016;7(1):1-6.

5. Al-Chalabi A, Hardiman O. The epidemiology of ALS: A conspiracy of genes, environment and time. *Nature Reviews Neurology*. 2013;9(11):617-628.

6. Hardiman O, Al-Chalabi A, Chio A, et al. Amyotrophic lateral sclerosis. *Nature reviews Disease primers*. 2017;3(1):1-19.

7. Oskarsson B, Horton DK, Mitsumoto H. Potential environmental factors in amyotrophic lateral sclerosis. *Neurologic Clinics*. 2015;33(4):877-888.

8. Longinetti E, Fang F. Epidemiology of amyotrophic lateral sclerosis: An update of recent literature. *Current Opinion In Neurology*. 2019;32(5):771.

9. Dominici F, Peng RD, Bell ML, et al. Fine particulate air pollution and hospital admission for cardiovascular and respiratory diseases. *JAMA*. 2006;295(10):1127-1134.

10. Bennett JE, Tamura-Wicks H, Parks RM, et al. Particulate matter air pollution and national and county life expectancy loss in the USA: A spatiotemporal analysis. *PLOS Medicine*. 2019;16(7):e1002856. doi:10.1371/journal.pmed.1002856

11. Schwartz J. Particulate air pollution and chronic respiratory disease. *Environmental Research*. 1993;62(1):7-13.

12. Schwartz J. The distributed lag between air pollution and daily deaths. *Epidemiology*. 2000;11(3):320-326.

13. Brook RD, Rajagopalan S, Pope III CA, et al. Particulate matter air pollution and cardiovascular disease: An update to the scientific statement from the American Heart Association. *Circulation*. 2010;121(21):2331-2378.

14. Dockery DW, Pope CA, Xu X, et al. An association between air pollution and mortality in six U.S. cities. *New England Journal of Medicine*. 1993;329(24):1753-1759. doi:10.1056/NEJM199312093292401

15. Block ML, Elder A, Auten RL, et al. The outdoor air pollution and brain health workshop. *Neurotoxicology*. 2012;33(5):972-984.

16. Zanobetti A, Dominici F, Wang Y, Schwartz JD. A national case-crossover analysis of the short-term effect of PM 2.5 on hospitalizations and mortality in subjects with diabetes and neurological disorders. *Environmental Health*. 2014;13(1):1-11.

17. Ritz B, Lee PC, Hansen J, et al. Traffic-related air pollution and Parkinson's disease in Denmark: A case–control study. *Environmental Health Perspectives*. 2016;124(3):351-356.

18. Kioumourtzoglou MA, Schwartz JD, Weisskopf MG, et al. Long-term PM2.5 exposure and neurological hospital admissions in the northeastern United States. *Environmental health perspectives*. 2016;124(1):23-29.

19. Levesque S, Surace MJ, McDonald J, Block ML. Air pollution & the brain: Subchronic diesel exhaust exposure causes neuroinflammation and elevates early markers of neurodegenerative disease. *Journal of Neuroinflammation*. 2011;8(1):1-10.

20. Heusinkveld HJ, Wahle T, Campbell A, et al. Neurodegenerative and neurological disorders by small inhaled particles. *Neurotoxicology*. 2016;56:94-106.

21. Power MC, Weisskopf MG, Alexeeff SE, Coull BA, Spiro III A, Schwartz J. Traffic-related air pollution and cognitive function in a cohort of older men. *Environmental Health Perspectives*. 2011;119(5):682-687.

22. Dubowsky SD, Suh H, Schwartz J, Coull BA, Gold DR. Diabetes, obesity, and hypertension may enhance associations between air pollution and markers of systemic inflammation. *Environmental Health Perspectives*. 2006;114(7):992-998.

23. Ruckerl R, Ibald-Mulli A, Koenig W, et al. Air pollution and markers of inflammation and coagulation in patients with coronary heart disease. *American Journal of Respiratory and Critical Care Medicine*. 2006;173(4):432-441.

24. Hoffmann B, Moebus S, Dragano N, et al. Chronic residential exposure to particulate matter air pollution and systemic inflammatory markers. *Environmental Health Perspectives*. 2009;117(8):1302-1308.

25. Kelly FJ. Oxidative stress: Its role in air pollution and adverse health effects. *Occupational and Environmental Medicine*. 2003;60(8):612-616.

26. Chuang KJ, Chan CC, Su TC, Lee CT, Tang CS. The effect of urban air pollution on inflammation, oxidative stress, coagulation, and autonomic dysfunction in young adults. *American journal of respiratory and critical care medicine*. 2007;176(4):370-376.

27. Li N, Sioutas C, Cho A, et al. Ultrafine particulate pollutants induce oxidative stress and mitochondrial damage. *Environmental Health Perspectives*. 2003;111(4):455-460.

28. Sørensen M, Daneshvar B, Hansen M, et al. Personal PM2.5 exposure and markers of oxidative stress in blood. *Environmental health perspectives*. 2003;111(2):161-166.

29. Block ML, Calderón-Garcidueñas L. Air pollution: Mechanisms of neuroinflammation and CNS disease. *Trends in neurosciences*. 2009;32(9):506-516.

30. Perry VH, Cunningham C, Holmes C. Systemic infections and inflammation affect chronic neurodegeneration. *Nature Reviews Immunology*. 2007;7(2):161-167.

31. Bergeron C. Oxidative stress: Its role in the pathogenesis of amyotrophic lateral sclerosis. *Journal of the neurological sciences*. 1995;129:81-84.

32. Mhatre M, Floyd RA, Hensley K. Oxidative stress and neuroinflammation in Alzheimer's disease and amyotrophic lateral sclerosis: Common links and potential therapeutic targets. *Journal of Alzheimer's disease*. 2004;6(2):147-157.

33. D'Amico E, Factor-Litvak P, Santella RM, Mitsumoto H. Clinical perspective on oxidative stress in sporadic amyotrophic lateral sclerosis. *Free radical biology and medicine*. 2013;65:509-527.

34. Perry VH, Nicoll JA, Holmes C. Microglia in neurodegenerative disease. *Nature Reviews Neurology*. 2010;6(4):193-201.

35. Nunez Y, Boehme AK, Weisskopf MG, et al. Fine particle exposure and clinical aggravation in neurodegenerative diseases in New York State. *Environmental Health Perspectives*. 2021;129(2):027003.

36. Nunez Y, Boehme AK, Li M, et al. Parkinson's disease aggravation in association with fine particle components in New York State. *Environmental Research*. 2021;201:111554.

37. Malek AM, Barchowsky A, Bowser R, et al. Exposure to hazardous air pollutants and the risk of amyotrophic lateral sclerosis. *Environmental Pollution*. 2015;197:181-186.

38. Yu Z, Peters S, van BL, et al. Long-Term Exposure to Ultrafine Particles and Particulate Matter Constituents and the Risk of Amyotrophic Lateral Sclerosis. *Environmental Health Perspectives*. 2021;129(9):097702. doi:10.1289/EHP9131

39. Seelen M, Toro CRA, Veldink JH, et al. Long-term air pollution exposure and amyotrophic lateral sclerosis in Netherlands: A population-based case–control study. *Environmental Health Perspectives*. 2017;125(9):097023. doi:10.1289/EHP1115

40. Nunez Y, Balalian A, Parks RM, et al. Exploring relevant time windows in the association between PM2.5 exposure and Amyotrophic Lateral Sclerosis: A case–control study in Denmark. *American Journal of Epidemiology*. Published online Under Revision.

41. Strak M, Weinmayr G, Rodopoulou S, et al. Long term exposure to low level air pollution and mortality in eight European cohorts within the ELAPSE project: Pooled analysis. *BMJ*. 2021;374:n1904. doi:10.1136/bmj.n1904

42. Hamra GB, Laden F, Cohen AJ, Raaschou-Nielsen O, Brauer M, Loomis D. Lung cancer and exposure to nitrogen dioxide and traffic: A systematic review and meta-analysis. *Environmental Health Perspectives*. 2015;123(11):1107-1112.

43. Chen H, Kwong JC, Copes R, et al. Living near major roads and the incidence of dementia, Parkinson's disease, and multiple sclerosis: A population-based cohort study. *The Lancet*. 2017;389(10070):718-726.

44. Gibson EA, Nunez Y, Abuawad A, et al. An overview of methods to address distinct research questions on environmental mixtures: An application to persistent organic pollutants and leukocyte telomere length. *Environmental Health*. 2019;18(1):1-16.

45. Frank L. When an entire country is a cohort. *Science*. 2000;287(5462):2398-2399.

46. Schmidt M, Schmidt SAJ, Sandegaard JL, Ehrenstein V, Pedersen L, Sørensen HT. The Danish National Patient Registry: A review of content, data quality, and research potential. *Clinical epidemiology*. 2015;7:449.

47. Trabjerg BB, Garton FC, van Rheenen W, et al. ALS in Danish registries: heritability and links to psychiatric and cardiovascular disorders. *Neurology Genetics*. 2020;6(2).

48. Mathis S, Goizet C, Soulages A, Vallat JM, Le Masson G. Genetics of amyotrophic lateral sclerosis: A review. *Journal of the Neurological Sciences*. 2019;399:217-226.

49. Kioumourtzoglou MA, Seals RM, Himmerslev L, Gredal O, Hansen J, Weisskopf MG. Comparison of diagnoses of amyotrophic lateral sclerosis by use of death certificates and hospital discharge data in the Danish population. *Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration*. 2015;16(3-4):224-229.

50. Pedersen CB. The Danish civil registration system. *Scandinavian journal of public health*. 2011;39(7_suppl):22-25.

51. Langholz B, Goldstein L. Risk set sampling in epidemiologic cohort studies. *Statistical Science*. Published online 1996:35-53.

52. Sillman S. The relation between ozone, NOx and hydrocarbons in urban and polluted rural environments. *Atmospheric Environment*. 1999;33(12):1821-1845.

53. Khan J, Kakosimos K, Raaschou-Nielsen O, et al. Development and performance evaluation of new AirGIS–a GIS based air pollution and human exposure modelling system. *Atmospheric environment*. 2019;198:102-121.

54. Brandt J, Christensen JH, Frohn LM, Palmgren F, Berkowicz R, Zlatev Z. Operational air pollution forecasts from European to local scale. *Atmospheric Environment*. 2001;35:S91-S98.

55. Brandt J, Christensen J, Frohn L, Berkowicz R. Air pollution forecasting from regional to urban street scale—-implementation and validation for two cities in Denmark. *Physics and Chemistry of the Earth, Parts A/B/C*. 2003;28(8):335-344.

56. Frohn LM, Ketzel M, Christensen JH, et al. Modelling ultrafine particle number concentrations at address resolution in Denmark from 1979-2018–Part 1: Regional and urban scale modelling and evaluation. *Atmospheric Environment*. 2021;264:118631.

57. Raaschou-Nielsen O, Andersen ZJ, Hvidberg M, et al. Lung cancer incidence and long-term exposure to air pollution from traffic. *Environmental health perspectives*. 2011;119(6):860-865.

58. Raaschou-Nielsen O, Sørensen M, Ketzel M, et al. Long-term exposure to traffic-related air pollution and diabetes-associated mortality: A cohort study. *Diabetologia*. 2013;56(1):36-46.

59. Sørensen M, Hoffmann B, Hvidberg M, et al. Long-term exposure to traffic-related air pollution associated with blood pressure and self-reported hypertension in a Danish cohort. *Environmental health perspectives*. 2012;120(3):418-424.

60. Seinfeld J, Pandis S. Atmospheric chemistry and physics. 1997. *New York*. Published online 2008.

61. Galvin M, Gaffney R, Corr B, Mays I, Hardiman O. From first symptoms to diagnosis of amyotrophic lateral sclerosis: Perspectives of an Irish informal caregiver cohort—a thematic analysis. *BMJ Open*. 2017;7(3). doi:10.1136/bmjopen-2016-014985

62. Dickerson AS, Hansen J, Kioumourtzoglou MA, Specht AJ, Gredal O, Weisskopf MG. Study of occupation and amyotrophic lateral sclerosis in a Danish cohort. *Occup Environ Med*. 2018;75(9):630-638. doi:10.1136/oemed-2018-105110

63. Roberts AL, Johnson NJ, Chen JT, Cudkowicz ME, Weisskopf MG. Race/ethnicity, socioeconomic status, and ALS mortality in the United States. *Neurology*. 2016;87(22):2300-2308.

64. Bucher BT, Shi J, Pettit RJ, Ferraro J, Chapman WW, Gundlapalli A. Determination of marital status of patients from structured and unstructured electronic healthcare data. In: *AMIA Annual Symposium Proceedings*. Vol 2019. American Medical Informatics Association; 2019:267.

65. Norman RE, Carpenter DO, Scott J, Brune MN, Sly PD. Environmental exposures: an underrecognized contribution to noncommunicable diseases. *Reviews on environmental health*. 2013;28(1):59-65.

66. Rothman KJ, Greenland S, Lash TL, others. *Modern Epidemiology*. Vol 3. Wolters Kluwer Health/Lippincott Williams & Wilkins Philadelphia; 2008.

67. Gelman A, Carlin JB, Stern HS, Dunson DB, Vehtari A, Rubin DB. *Bayesian Data Analysis, Third Edition*. CRC Press; 2013.

68. Mostofsky E, Schwartz J, Coull BA, et al. Modeling the association between particle constituents of air pollution and health outcomes. *American journal of epidemiology*. 2012;176(4):317-326.

69. Thunis P, Degraeuwe B, Pisoni E, et al. PM2.5 source allocation in European cities: A SHERPA modelling study. *Atmospheric Environment*. 2018;187:93-106.

70. Nuvolone D, Petri D, Voller F. The effects of ozone on human health. *Environmental Science and Pollution Research*. 2018;25(9):8074-8088.

71. Martin R, Peters G, Wilkinson J. Symmetric decomposition of a positive definite matrix. *Numerische Mathematik*. 1965;7(5):362-383.

72. Polson NG, Scott JG. On the half-Cauchy prior for a global scale parameter. *Bayesian Analysis*. 2012;7(4):887-902.

73. Gelman A. Prior distributions for variance parameters in hierarchical models (comment on article by Browne and Draper). *Bayesian Anal*. 2006;1(3):515-534. doi:10.1214/06-BA117A

74. Lewandowski D, Kurowicka D, Joe H. Generating random correlation matrices based on vines and extended onion method. *Journal of multivariate analysis*. 2009;100(9):1989-2001.

75. Gelman A, Rubin DB. Inference from iterative simulation using multiple sequences. *Statistical science*. 1992;7(4):457-472.

76. R Core Team. R: A language and environment for statistical computing. Published online 2013.

77. Povedano M, Saez M, Martinez-Matos JA, Barceló MA. Spatial assessment of the association between long-term exposure to environmental factors and the occurrence of amyotrophic lateral sclerosis in Catalonia, Spain: A population-based nested case–control study. *Neuroepidemiology*. 2018;51(1-2):33-49.

78. von Schneidemesser E, Mar KA, Saar D. Black carbon in Europe: Targeting an air Pollutant and climate forcer. Published online 2017.

79. Pamphlett R, Rikard-Bell A. Different occupations associated with amyotrophic lateral sclerosis: Is diesel exhaust the link? *PloS One*. 2013;8(11):e80993.

80. Zhang R, Dai Y, Zhang X, et al. Reduced pulmonary function and increased pro-inflammatory cytokines in nanoscale carbon black-exposed workers. *Part Fibre Toxicol*. 2014;11:73. doi:10.1186/s12989-014-0073-1

81. Gao X, Xu H, Shang J, et al. Ozonized carbon black induces mitochondrial dysfunction and DNA damage. *Environ Toxicol*. 2017;32(3):944-955. doi:10.1002/tox.22295

82. Kyjovska ZO, Jacobsen NR, Saber AT, et al. DNA damage following pulmonary exposure by instillation to low doses of carbon black (Printex 90) nanoparticles in mice. *Environ Mol Mutagen*. 2015;56(1):41-49. doi:10.1002/em.21888

83. Benatar M, Turner MR, Wuu J. Defining pre-symptomatic amyotrophic lateral sclerosis. *Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration*. 2019;20(5-6):303-309.

84. Benatar M, Wuu J, McHutchison C, et al. Preventing amyotrophic lateral sclerosis: insights from pre-symptomatic neurodegenerative diseases. Published online 2021.

85. Eisen A, Kiernan M, Mitsumoto H, Swash M. Amyotrophic lateral sclerosis: A long preclinical period? *Journal of Neurology, Neurosurgery & Psychiatry*. 2014;85(11):1232-1238.

86. Weisskopf MG, Webster TF. Trade-offs of personal vs. more proxy exposure measures in environmental epidemiology. *Epidemiology (Cambridge, Mass)*. 2017;28(5):635.

87. Carroll RJ, Ruppert D, Stefanski LA, Crainiceanu CM. *Measurement Error in Nonlinear Models: A Modern Perspective*. CRC press; 2006.

88. Frisch M, Simonsen J. Marriage, cohabitation and mortality in Denmark: national cohort study of 6.5 million persons followed for up to three decades (1982–2011). *International Journal of Epidemiology*. 2013;42(2):559-578.

89. Bobb JF, Valeri L, Claus Henn B, et al. Bayesian kernel machine regression for estimating the health effects of multi-pollutant mixtures. *Biostatistics*. 2015;16(3):493-508.

Figure Captions

Figure 1. Spearman correlation of 1,- 5-, and 10-year average pollutant concentrations.

Figure 2. Percentage change in odds of ALS diagnosis per 1-, 5- and 10-year average standard deviation (SD) increase for each pollutant. Results are from the Bayesian hierarchical model including each of elemental carbon (EC), nitrogen oxides (NO_x), carbon monoxide (CO), non-elemental carbon fine particles (non-EC $PM_{2.5}$), and ozone (O_3)together, and were additionally adjusted by age, sex, year of birth, vital status, socioeconomic status, civil status, last reported place of residence, and place of birth.

Table 1. Demographic characteristics of cases and controls for 5-year average exposure group.

Characteristic	Overall, N = 23,232	Case, N = 3,934	Control, N = 19,298
Average age (years)	66 (12)	66 (12)	66 (12)
Sex, n(%)			
Female	10,973 (47%)	1,854 (47%)	9,119 (47%)
Male	12,259 (53%)	2,080 (53%)	10,179 (53%)
Socioeconomic status (SES), n(%)			
Group 1 (Highest)	2,337 (10%)	451 (11%)	1,886 (9.8%)
Group 2	2,839 (12%)	499 (13%)	2,340 (12%)
Group 3	4,360 (19%)	785 (20%)	3,575 (19%)
Group 4	6,598 (28%)	1,076 (27%)	5,522 (29%)
Group 5 (Lowest)	4,419 (19%)	717 (18%)	3,702 (19%)
Group 9 (Unknown)	2,679 (12%)	406 (10%)	2,273 (12%)
Place of birth, n(%)			
Greater Copenhagen	4,858 (21%)	831 (21%)	4,027 (21%)
Big cities of Denmark	7,923 (34%)	1,357 (34%)	6,566 (34%)
Rest of Denmark	9,009 (39%)	1,548 (39%)	7,461 (39%)
Greenland	243 (1.0%)	53 (1.3%)	190 (1.0%)
Foreign	1,065 (4.6%)	122 (3.1%)	943 (4.9%)
Unknown	134 (0.6%)	23 (0.6%)	111 (0.6%)
Civil status, n(%)			
Married	14,158 (61%)	2,411 (61%)	11,747 (61%)
Divorced	2,703 (12%)	433 (11%)	2,270 (12%)
Widowed	4,224 (18%)	726 (18%)	3,498 (18%)
Never married	2,147 (9.2%)	364 (9.3%)	1,783 (9.2%)
Last reported place of residence, n(%)			
Greater Copenhagen	7,778 (33%)	1,328 (34%)	6,450 (33%)
Big cities of Denmark	3,703 (16%)	618 (16%)	3,085 (16%)
Rest of Denmark	11,747 (51%)	1,988 (51%)	9,759 (51%)
Greenland	4 (<0.1%)	0 (0%)	4 (<0.1%)

Table 2. Summary of 1,- 5-, and 10-year average pollutant concentrations of elemental carbon (EC), nitrogen oxides (NO_x), carbon monoxide (CO), non-elemental carbon fine particles (non-EC $PM_{2.5}$), and ozone (O_3) (all mean [SD] in $\mu g/m^3$).

	Pollutant	Overall, N = 23,270	Case, N = 3,937	Control, N = 19,333
1-year average	EC	0.81 (0.42)	0.83 (0.44)	0.81 (0.42)
	NO_X	26 (19)	26 (20)	26 (19)
	CO	224 (97)	226 (101)	224 (96)
	non-EC $PM_{2.5}$	11.17 (2.32)	11.20 (2.34)	11.17 (2.31)
	O_3	52.6 (6.1)	52.4 (6.2)	52.6 (6.1)
	Pollutant	Overall, N = 23,232	Case, N = 3,934	Control, N = 19,298
5-year average	EC	0.85 (0.42)	0.86 (0.45)	0.85 (0.42)
	NO_X	27 (20)	28 (21)	27 (20)
	CO	238 (106)	239 (112)	237 (105)
	non-EC $PM_{2.5}$	11.76 (2.37)	11.78 (2.41)	11.76 (2.37)
	O_3	51.9 (6.0)	51.9 (6.1)	52.0 (6.0)
	Pollutant	Overall, N = 23,179	Case, N = 3,929	Control, N = 19,250
10-year average	EC	0.89 (0.43)	0.89 (0.46)	0.88 (0.43)
	NO_X	29 (20)	29 (22)	29 (20)
	CO	253 (115)	255 (122)	253 (113)
	non-EC $PM_{2.5}$	12.53 (2.55)	12.55 (2.59)	12.52 (2.55)
	O_3	51.3 (6.0)	51.3 (6.1)	51.4 (6.0)

Figure 1

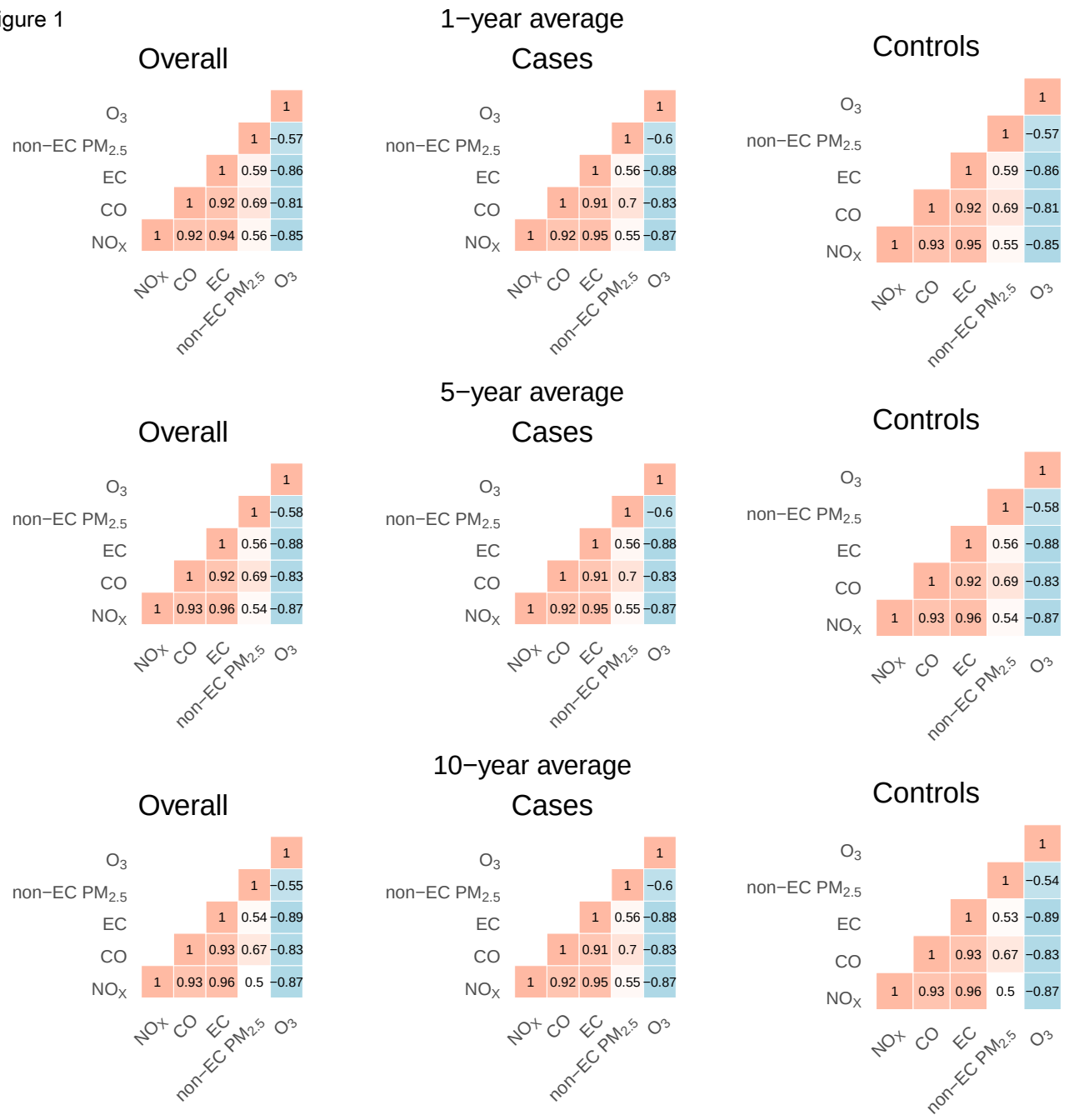
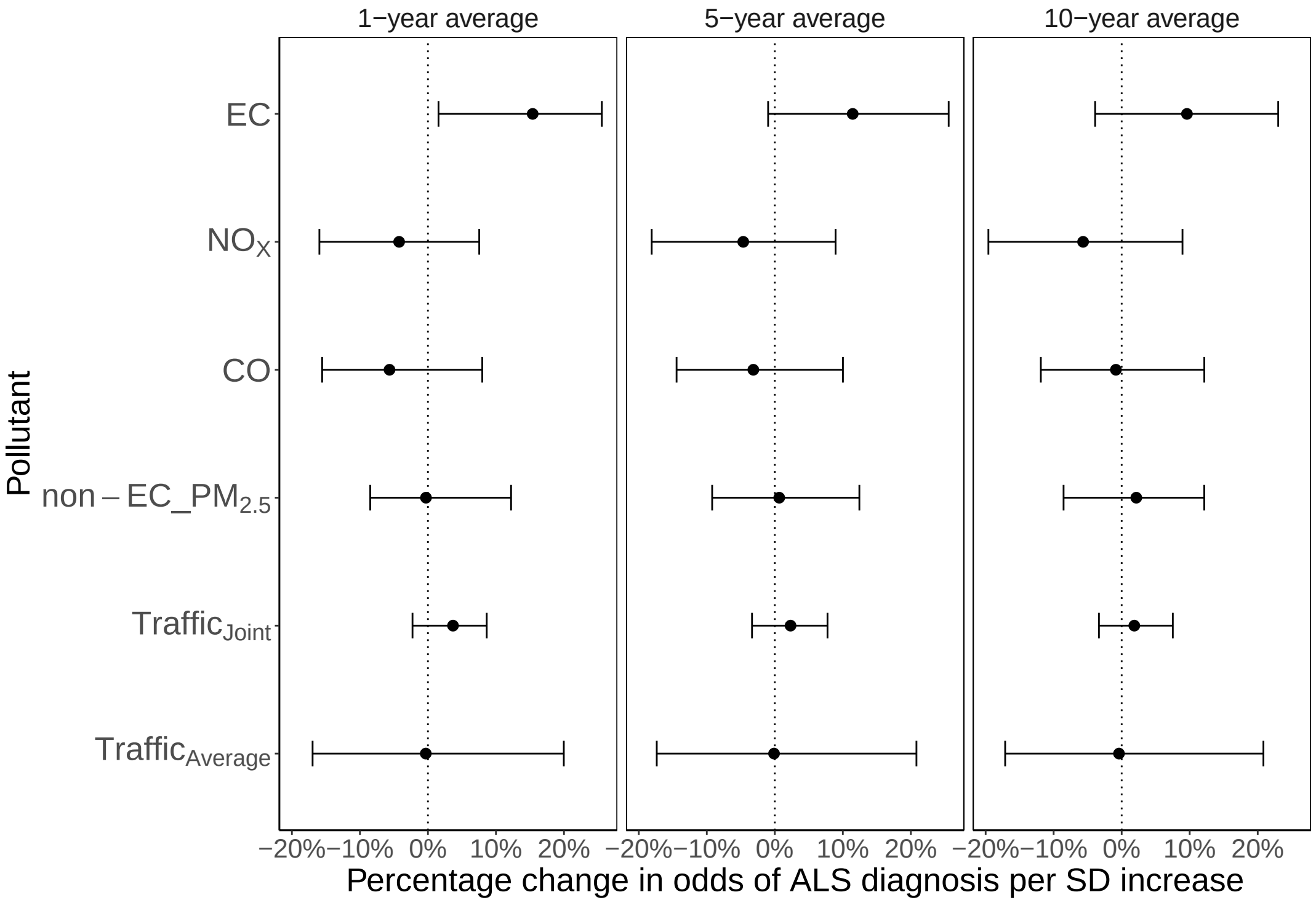

1−year average
Overall
Cases
Controls
5−year average
Overall
Cases
Controls
10−year average
Overall
Cases
Controls
O3
non−EC PM2.5
EC
CO
NOX
1
0.92
0.94
0.56
−0.85
0.59
−0.86
0.69
−0.81
−0.57
−0.6
0.91
0.7
−0.83
0.95
0.55
−0.87
0.93
0.96
0.54
−0.58
0.56
−0.88
0.69
−0.83
−0.87
0.67
−0.83
0.5
−0.87
−0.55
0.54
−0.89
0.53
−0.89
−0.54

Figure 2


1−year average
5−year average
10−year average
Pollutant
EC
NOX
CO
non − EC_PM2.5
TrafficJoint
TrafficAverage
−20%
−10%
0%
10%
20%
Percentage change in odds of ALS diagnosis per SD increase

Click here to access/download

Supplemental Digital Content

supplemental digital content.pdf

Click here to access/download

Supplemental Digital Content

supplemental digital content.pdf

Item not required

Main Text with highlights showing changes

Item not required