Epidemiology

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

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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 3,939 ALS cases from the Danish National Patient Register diagnosed during 1989–2013 and matched on age, sex, year of birth and vital status to 19,298 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 a Bayesian hierarchical conditional logistic model, adjusting for potential confounders, to estimate individual pollutant associations, well as joint and average associations for the 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 being individually associated with an increase in odds (11.5%; 95% credible interval[CrI]:-1.0%,25.6%; 96.3% posterior probability of a 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 a positive association), CO (SD=106μg/m^3) (-3.2%;95%CrI-14.4%,10.0%; 26.7% posterior probability of a 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: A high probability of a positive association between ALS diagnosis and EC concentration, though results are inconclusive. Further work is needed to understand the role of traffic-related air pollution on ALS pathogenesis.
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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

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

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

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

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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 3,939 ALS cases from the Danish National Patient Register diagnosed during 1989–2013 and matched on age, sex, year of birth and vital status to 19,298 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 a Bayesian hierarchical conditional logistic model, adjusting for potential confounders, to estimate individual pollutant associations, well as joint and average associations for the 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 being individually associated with an increase in odds (11.5%; 95% credible interval[CrI]:-1.0%,25.6%; 96.3% posterior probability of a 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 a positive association), CO (SD=106μg/m^3) (-3.2%;95%CrI-14.4%,10.0%; 26.7% posterior probability of a 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: A high probability of a positive association between ALS diagnosis and EC concentration, though results are inconclusive. Further work is needed to understand the role of traffic-related air pollution on 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 modelling 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 three 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 toxicological studies also support several plausible biological 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] 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 modelling 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.

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 January 1st 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 modelling 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 modelling system for traffic pollution, developed for application in Danish air pollution epidemiological studies. The modelling system integrates air pollution dispersion models, digital maps, national and local administrative databases, concentrations of air pollutants at regional, urban background and street level, meteorological data, and a Geographic Information System (GIS). The modelling 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.[17,57–59] The models have good predictive accuracy, with average monthly correlations between measured and modelled 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 modelling 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 one 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 increase in each of EC, NO_x, CO), and (c) an average traffic association (i.e., average percentage change in odds

of ALS diagnosis from 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 modelled the log-odds of ALS diagnosis, as follows:

$$logit[\Pr(Y_{ci} = 1)] = \alpha_c +$$

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

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

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

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); β_{EC}, β_{NO_x}, β_{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:

$$\beta_{Traffic} = [\beta_{EC}, \beta_{\mathrm{NO}_x}, \beta_{CO}],$$

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

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

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

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 modelling 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 3,939 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.

For 5-year average pollutant concentrations, we observed the largest overall association 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%). 1-year EC average exposure was associated with an increase in odds of ALS diagnosis (15.4%; 95% CrI: 1.6%, 25.6%). 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 D) overlapped with the credible intervals of the EC

term in the multi-pollutant models (eFigure 1; models B, 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%). 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 1). A map 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) is also available in eFigure 2.

Discussion

In the largest case-control study of ALS and traffic-related air pollution to date, 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. Nevertheless, the consistency of the sign of the central estimate of EC in all models suggests that EC may be a driver of the ALS

and traffic-related pollutant association, 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 (0.95 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 used one the largest number 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, however, are not expected to cause the predicted pollutant concentrations, given exposure assessment. 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 modelled exposure will be inaccurate to some degree. However, any error is not likely correlated with ALS diagnosis, and therefore any bias would be towards 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 (BKMR)[89] might be useful in further exploring the robustness of joint associations in a different framework, though BKMR was not appropriate for our particular research question, since BKMR 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.

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

Characteristic	Overall, N = 23,232[a]	Case, N = 3,934[a]	Control, N = 19,298[a]
Average age (years)	66 (12)	66 (12)	66 (12)
Sex			
Female	10,973 (47%)	1,854 (47%)	9,119 (47%)
Male	12,259 (53%)	2,080 (53%)	10,179 (53%)
Socioeconomic status (SES)			
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			
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			
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			
Greater Copenhagen	1,887 (8.1%)	335 (8.5%)	1,552 (8.0%)
Big cities of Denmark	9,385 (40%)	1,590 (40%)	7,795 (40%)
Rest of Denmark	11,954 (51%)	2,008 (51%)	9,946 (52%)
Greenland	6 (<0.1%)	1 (<0.1%)	5 (<0.1%)

[a]Mean (SD); n (%)

Table 2. Summary of 5-year average pollutant concentrations (all in μg/m^3).

Pollutant	Overall, N = 23,232[a]	Case, N = 3,934[a]	Control, N = 19,298[a]
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 PM2.5	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)

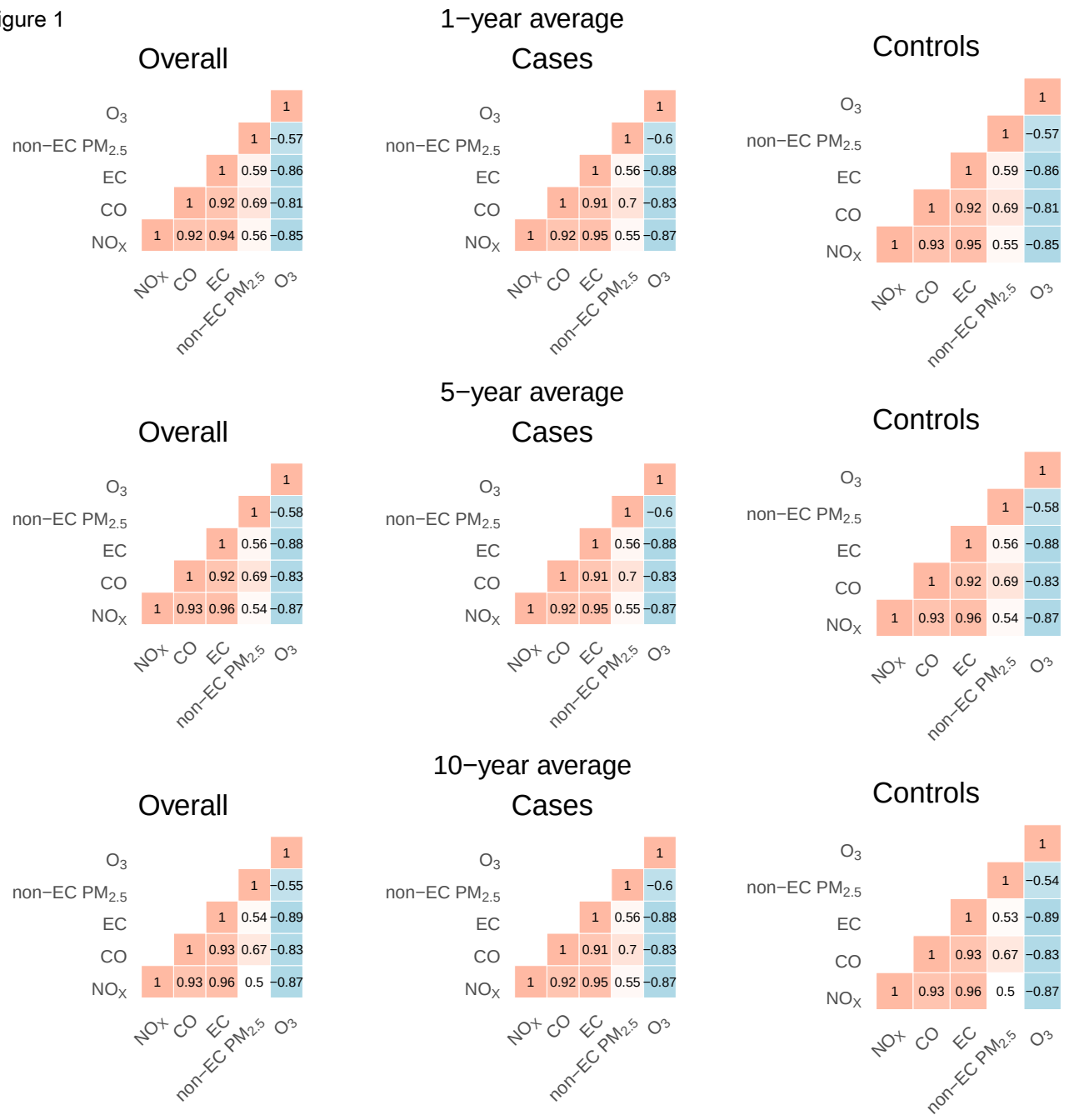
[a]Mean (SD)

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 EC, NO_x CO, and non-EC $PM_{2.5}$ 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.

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
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−0.87
0.93
0.96
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−0.58
0.56
−0.88
0.69
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−0.87
0.67
−0.83
0.5
−0.87
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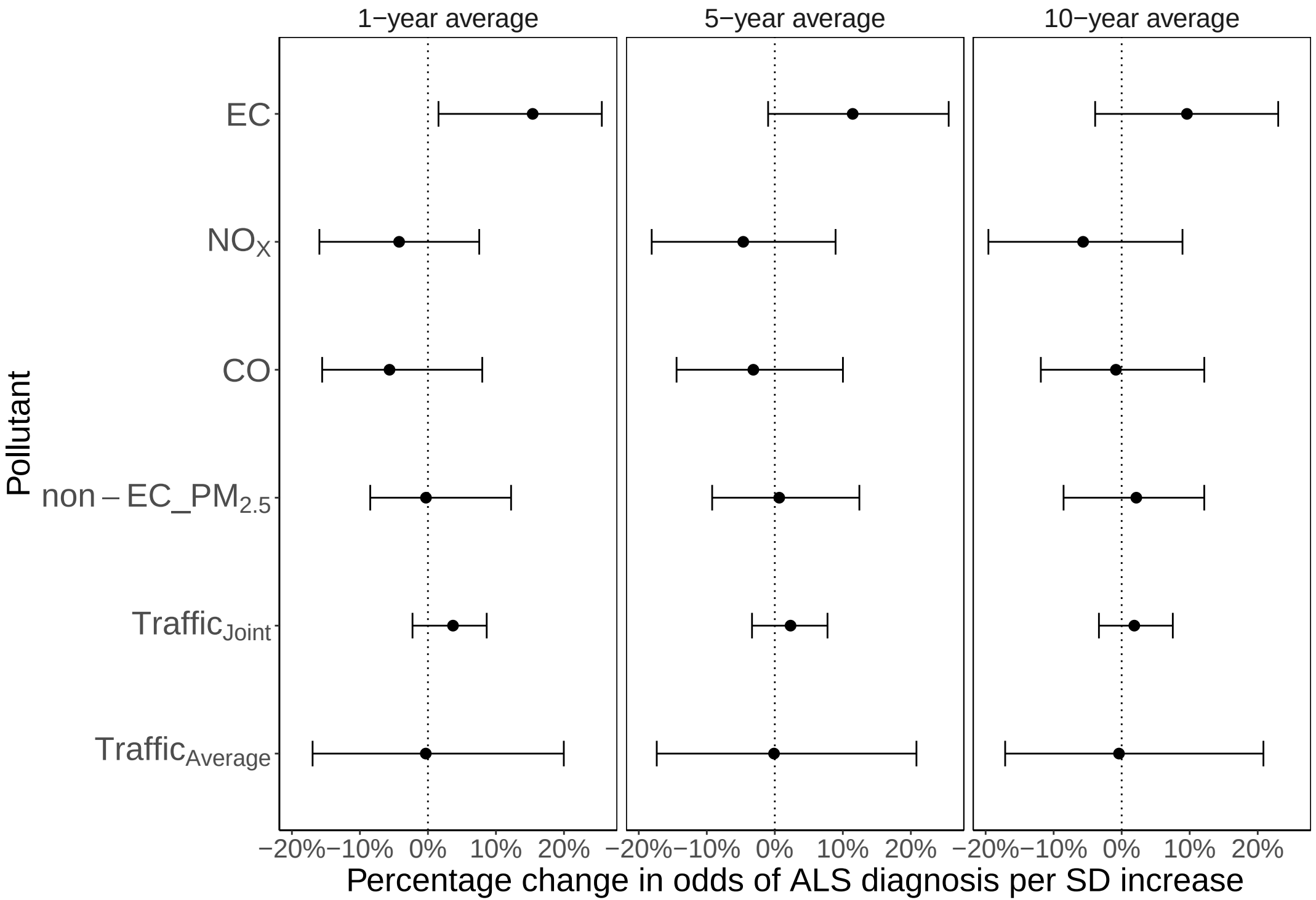
Figure 2


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

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Comments from the editors

We thank the Editors and Reviewers for their thoughtful and constructive suggestions. We have revised the manuscript in response to their comments, as detailed below.

All page/line/reference numbers refer to the clean revised manuscript.

Comments from reviewers and editors' concerns

1.Thank you for your response to our follow-up e-mail regarding the Nunez et al. paper. We consider this to be a closely-related work, given the overlap in study design and data utilized, and that the two articles share the same overarching goal of understanding the impact of air pollution on ALS. We do recognize the distinction in the message of each paper and methods employed. We offer authors clear guidance on close-related works (see https://edmgr.ovid.com/epid/accounts/ifauth.htm, under Essential Conditions). To abide by this guidance, and also provide clearer perspectives on this research to readers, please include reference to the Nunez et al. article (including as a placeholder in the reference list) and add text to set the two papers apart. In fact, the Nunez et al. article, with its focus on PM2.5 and lag structure, seems to set the stage for your current paper, including the selection of exposure averaging times and potentially interpretation of findings, given that PM2.5 EC appears to be the driver of the observed effects in your paper.

We understand and appreciate the Editors' concerns. We now reference the Nunez et al. article (currently under review) in the Introduction of the revised manuscript as a foundation for this study in the revised manuscript (PP. 3-4, Lines 66-69):

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]

We have now also adapted the Discussion to clarify how our results are consistent with the other paper in the revised manuscript (P. 15, Lines 327-330):

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.*

2. The revised manuscript is much clearer in your goal of estimating individual, joint, and average (or overall) associations for the three traffic pollutants. However, the specific definitions need additional clarification, and the ordering and terminology used should be consistent throughout:

a) for definitions, those provided on page 8, lines 172-176, are helpful, but do not fully clarify the difference between joint and overall average effects. The joint effect is expressed as the percent change in odds of ALS diagnosis with a simultaneous increase in each of the three traffic pollutants. For the overall effect, is this the average percent change in the odds of ALS diagnosis with an increase in each of the three traffic pollutants

independently? Please clarify the wording in this section. Furthermore, if I am understanding the definition of the 'overall' effect correctly, this may be best termed as the 'average' effect throughout the manuscript, since this more closely aligns with the definition applied (Figure 2 already uses 'Traffic_average'). Similar clarification should also be made on line 216 (i.e., refer to average association across the three traffic pollutants).

The Editor is correct. We appreciate the suggestion. To clarify, we have changed the terms used in the definition of the average effect from 'overall' to 'average' throughout the revised manuscript, e.g., (PP. 8-9, Lines 180-185):

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 increase in each of EC, NO_x, CO), and (c) an average traffic association (i.e., average percentage change in odds of ALS diagnosis from each of EC, NO_x, CO), while accounting for the variance-covariance structure between the highly-correlated exposures and their coefficients.[67]

b) for ordering, please discuss the types of associations in the same order consistently throughout (e.g., including in abstract methods section lines 12-13, and suggest reordering Figure 2 to this order as well). I.e., referring to individual effects, joint effects, average effects in this order as is presented in the methods.

We have done this in the Methods section of the Abstract in the revised manuscript (P. 1, Lines 11-14):

We used a Bayesian hierarchical conditional logistic model, adjusting for potential confounders, to estimate individual pollutant associations, well as joint and average associations for the traffic-related pollutants (EC, NO_x, CO).

We have also maintained this order throughout the rest of the revised manuscript, e.g., (P. 4, Lines 73-77):

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.

We have re-ordered Figure 2 accordingly in the revised manuscript (as below):

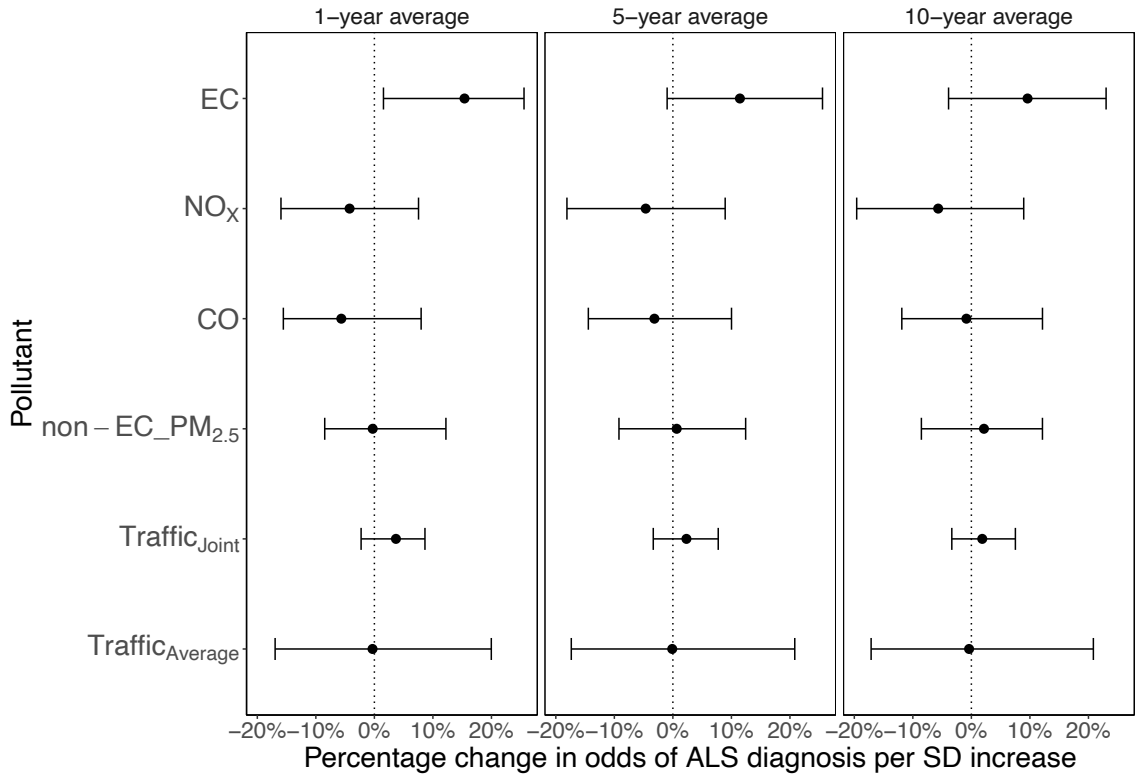

Figure 2. Percentage change in odds of ALS diagnosis per 1-, 5- and 10-year average standard deviation (SD) increase for each pollutant. Results are from the Bayesian hierarchical model including each of EC, NO_x CO, and non-EC $PM_{2.5}$ together, and were additionally adjusted by age, sex, year of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

c) for terminology, the use of 'individual pollutant' effects should be used consistently throughout (e.g., in place of pollutant-specific, which currently appears in the abstract and several other places)

We have changed 'pollutant-specific' to 'individual pollutant' throughout the revised manuscript, e.g., (P. 10, Lines 211-212):

In our model, β_{EC}, β_{NO_x}, and β_{CO} represent the independent individual pollutant associations with ALS diagnosis.

3. Use of the phrase 'potential associations' in the abstract (lines 16 and 24) and discussion (line 297) is unclear and does not appear to fully describe the actual results of the analysis. It seems that you are using this word to describe associations that had a high probability of being truly positive. I suggest rewording these sections for clarity.

We have changed 'potential associations' to discussing the high probability of an association through the revised manuscript, e.g., (P. 14, Lines 307-311):

In the largest case-control study of ALS and traffic-related air pollution to date, 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.

4. The abstract results section needs cleaning up. The wording in general is imprecise, in particular the last sentence referring to probabilities.

We have clarified the Results section of the Abstract, maintaining the order from Points 2 and 3 above in the revised manuscript (P. 1, Lines 16-23):

Results: *For a standard deviation (SD) increase in 5-year average concentrations, EC (SD=0.42μg/m^3) had a high probability of being individually associated with an increase in odds (11.5%; 95% credible interval[CrI]:-1.0%,25.6%; 96.3% posterior probability of a 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 a positive association), CO (SD=106μg/m^3) (-3.2%;95%CrI-14.4%,10.0%; 26.7% posterior probability of a 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.*

5. Reviewer 1 had asked you to provide some additional information on the air pollution modeling system. While the system has been previously developed and validated, and also used in other epidemiological applications, it would be helpful to include a brief summary of how the models are developed. While it's interesting to know that air quality levels can be estimated efficiently by the system, it is more informative for readers to understand the underlying structure of the models. E.g., were these chemical transport models taking emissions and weather data into account? Were these outputs fused and/or calibrated with monitoring data?

We have added further details of DEHM-UBM-AirGIS, focusing more on the details of the model, in this section of the revised manuscript (P. 6, Lines 121-128):

In brief, DEHM-UBM-AirGIS is a human exposure modelling system for traffic pollution, developed for application in Danish air pollution epidemiological studies. The modelling system integrates air pollution dispersion models, digital maps, national and local administrative databases, concentrations of air pollutants at regional, urban background and street level, meteorological data, and a Geographic Information System (GIS). The modelling 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.

6. Lines 124-127 - from your response to reviewer comments, it appears that the subtraction of EC from PM2.5 was appropriate here given how PM2.5 was estimated by the air pollution modeling system (i.e., constructed from individual components). This is important to point out, as the simple subtraction of EC from PM2.5 when using monitoring data, for example, can cause errors given different measurement approaches for each.

We have added to the justification of how we recovered non-EC $PM_{2.5}$ in the revised manuscript (P. 6, Lines 132-136):

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 modelling system constructed $PM_{2.5}$ concentrations by adding from specific species of pollutants, one of which was EC.[53–56]

7. Line 303-304 - suggested change from 'would have been' to 'were'

We have done this in the revised manuscript (P. 14, Lines 314-315):

Overall conclusions for the association between EC and ALS diagnosis were similar from the single- or multi-pollutant models.

8. Lines 305-309 - since the EC effects were consistent across the single and multi-pollutant modeling, it seems that the conclusion of EC being a driver of the traffic-ALS association is supported. However, the point about the model having had 'limited success in identifying each individual pollutant's association with ALS' is unclear. Even though NO2 and CO may have had positive associations in their single-pollutant models, there could be reasons why EC effects predominated in the multi-pollutant context (either more relevant for ALS or less measurement error than the other pollutants). Please clarify.

We have clarified in the Discussion to suggest that the association of EC concentrations with ALS diagnosis was consistent, along with inconsistent associations for NO_x and CO, suggest that EC concentrations may have been most relevant in the revised manuscript (P. 14, Lines 315-317):

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.

9. Line 309 - suggested change from 'analysis' to 'study'

We have done this in the revised manuscript (PP. 14-15, Lines 317-319):

Nevertheless, the consistency of the sign of the central estimate of EC in all models suggests that EC may be a driver of the ALS and traffic-related pollutant association, though further study is required.

10. Line 353 on residual confounding - in the revision, you deleted a section on BMI and smoking, as per Reviewer 1 and gave responses to Reviewer 2 on this point. It is still not clear to me how factors such as BMI or smoking are not potential confounders in this analysis. A confounder is a factor that is a risk factor for the outcome, and a factor that is associated with the exposure. BMI and smoking would not be expected to 'cause' the exposure in this case, however, these factors could be geographically correlated in a way not captured by SES. It is not clear to me that SES necessarily blocks all potential confounding by these other factors and it would be helpful to include some additional discussion on this.

The Editor is correct that although BMI and smoking cannot cause the predicted pollutant concentrations, given the current exposure assessment method, they can be correlated with

them through neighborhood-level SES. It is possible that there may be some residual confounding by SES, in which case further adjusting our models for BMI and smoking status may have helped address this potential concern. However, information on individual-level BMI and smoking status is not available in the Danish Civil Registration System. Given the Reviewers' and Editor's comments, we have adjusted the Limitations paragraph accordingly

We have explained below in the revised manuscript (P. 17, Lines 366-372):

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, however, are not expected to cause the predicted pollutant concentrations, given exposure assessment. 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.

11. Line 367 - was the use of BKMR not appropriate for your research question because it currently does not accommodate application in case-control studies? In this case, I suggest using 'since BKMR is currently not available for case-control study applications'.

We have updated the language here per the Editor's suggestion in the revised manuscript (P. 17, Lines 382-385):

Other mixture model methods, such as Bayesian Kernel Machine Regression (BKMR)[89] might be useful in further exploring the robustness of joint associations in a different framework, though BKMR was not appropriate for our particular research question, since BKMR is currently not available for case-control study applications.

*** * * * ***

Preparing a revision

1. For estimates of causal effects, we strongly discourage the use of categorized P-values and language referring to statistical significance, including whether a confidence interval covers the null. We prefer instead interval estimation, which conveys the precision of the estimate with respect to sampling variability. We are more open to testing with respect to modeling decisions, such as for tests of interaction and for tests for trend.

We have avoided p-values throughout.

2. We do not permit acronyms unless they are generally recognized by epidemiologists (e.g. HIV is okay, but LVA is not). When in doubt, we recommend that you spell out.

We have been careful to introduce acronyms where used.

3. Please do not include uninformative precision (excessive decimal places). For example, percents should be rounded to nn%, n.n%, or 0.0n% and risk ratios should be rounded to nn, n.n, or 0.nn unless clarity of the presentation and the sample size justify more significant digits.

We have done this.

4. Please be sure to include explicit information about approval of human subjects research by an independent review board. If no such review was required, include an explicit statement about why the requirement for review was waived.

We have done this in the manuscript (P. 5, Lines 112-113):

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

5. Do not include public health policy recommendations in Brief Reports or Original Articles that present new research findings.

We have not included any public health policy recommendations.

6. Data appearing in the abstract must also be cited in the main text, not just in tables or figures.

We have done this.

7. Resubmissions must adhere to word limits. The word limits for main text (generally the introduction, methods, results, and discussion) are 1500 words for Brief Reports (plus 150 words for its abstract), 4000 words for Original Articles (plus 250 words for its abstract), 5000 words for reviews (plus 250 words for its abstract), 2000 words for Commentaries (no abstract), 600 words for Research Letters (no abstract), and 400 words for Letters to the Editor (no abstract).

We have done this, with an Abstract of 250 words and an Original Article of 4,120 words in the revised manuscript.

8. We advise that total word counts for Original Articles should not exceed 7500 words and for Brief Reports should not exceed 3500 words. The total word count includes main text (introduction, methods, results, and discussion), bibliography, figure legends, tables, and figures (250 words per figure, including each figure in a panel). The title page, abstract, acknowledgments, and funding information do not count in the total word count.

We have adhered to this, with a total word count of 6,643 words in the revised manuscript.

9. Figure labels: Make font size as large as possible, so as to be legible when figures are reduced for publication (typically one column [8.5cm] in width).

We have made the Figure labels large and legible.

10. Footnotes to tables and figures should use superscript lowercase letters to link content to the footnote, not symbols or numerals.

The footnote in Table 1 uses a superscript lowercase letter.

11. Do not use parenthetical phrases like "(data not shown), (results not shown), or (available from the authors upon request)." In these circumstances, the data or results should be provided in Supplementary Digital Content.

We have avoided any use of these phrases.

12. Additional details regarding submission requirements can be found in the Instructions for Authors, which are posted at http://edmgr.ovid.com/epid/accounts/ifauth.htm .

We have reviewed these details.

Preparing for resubmission

13. Prepare a response document for the Editor that responds point-by-point to the reviewers' comments (presenting each comment followed by your response). Give the page number where revised text can be found and, where practical, paste revised text directly into the reply document.

We have done this.

14. Submit versions of the manuscript with and without your changes displayed.

We have submitted clean and tracked versions of the revised manuscript.

15. Supplementary Digital Content should be submitted as a single PDF file, and you should use our convention - e.g. eFigure 1, eAppendix 2 - to label and refer to online content.

We have done this.

16. Authors should submit copies of any closely related manuscripts (published, in press, or under review).

As discussed with the Editors, we now mention the Nunez et al. paper into our revised manuscript.

17. Please revisit information about page charges and color printing charges available in the Instructions for Authors, which are posted at http://edmgr.ovid.com/epid/accounts/ifauth.htm .

We acknowledge the charges on the link provided.

18. We request that the complete revised manuscript (with all tables and figures) be completed by 14 Sep 2022. If you are not able to meet this deadline, please notify the editorial office.

We have submitted before 14th September 2022.

Resubmitting via Editorial Manager

19. Log-in to Editorial Manager as an author using the credentials above.
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Thank you for the resubmission instructions. We have followed them.

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 3,939 ALS cases from the Danish National Patient Register diagnosed during 1989–2013 and matched on age, sex, year of birth and vital status to 19,298 population-based controls free of ALS at index date. We used validated predictions of elemental carbon (EC), nitrogen oxides (NO_x), carbon monoxide (CO~~), elemental carbon (EC~~), 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 a Bayesian hierarchical conditional logistic model, adjusting for potential confounders, to estimate ~~overall and~~ individual pollutant associations, well as joint and average associations for the traffic-related pollutants (EC, NO_x, CO~~, EC), and pollutant-specific associations.~~).

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

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pollution~~. There was a 77.8% posterior probability of a positive association between the joint effect of pollutants and ALS diagnosis, 96.3% for EC, 27.8% for NO_x and 26.7% for CO.~~ concentrations.

Conclusions: A ~~potential~~high probability of a positive association between ALS diagnosis and ~~pollutants, particularly for EC~~EC concentration, though results are inconclusive. Further work is needed to understand the role of traffic-related air pollution on 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 modelling 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

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47	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 three 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 toxicological studies also support several plausible biological 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] ~~No study has hitherto attempted to understand the combined and individual associations of the pollutants in a single model. Air pollutants have been consistently associated with adverse health, primarily in single pollutant analyses.[13,17,40–42] However, they are~~

~~highly correlated with one another.[40] It is therefore a mixture modelling challenge to infer the association of multiple air pollutants and health outcomes.[43] Using three air pollutants commonly used in health studies as traffic related emissions tracers – nitrogen oxides (NO_x), carbon monoxide (CO), and elemental carbon (EC) – 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) overall~~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] 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 modelling 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.

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.[~~44~~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.[45][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,[46][47] and (ii) the very few juvenile ALS cases have been explained to a much larger degree by genetic mutations (~40%).[47][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%.[48][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.[49][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.[50][51]

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We obtained all addresses of cases and controls from January 1st 1979 onwards from the Danish Civil Registration System,[49] ~~including the dates of moving to and from each address, before the index date.~~[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 ~~the~~ Columbia University and the Danish Data Protection Agency.

Exposure data

~~We obtained predictions on monthly concentrations of nitrogen oxides (NO_x), carbon monoxide (CO), elemental carbon (EC), 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[51]), at residential addresses of study participants from the validated spatio-temporal air pollution modelling system DEHM-UBM-AirGIS that provides full space and time coverage over the study period, described in detail elsewhere.[52–55] In brief, DEHM-UBM-AirGIS is a human exposure modelling system for traffic pollution, developed for application in Danish air pollution epidemiological studies. The modelling system is 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.[17,56–58] The models have good predictive accuracy, with average monthly correlations between measured and modelled results of~~

~~0.85 for NO_x, 0.91 for CO, 0.92 for O_3, 0.79 for EC, and 0.83 for annual concentrations of $PM_{2.5}$.[52,55] Because traffic is a major source of $PM_{2.5}$ and EC one of the main $PM_{2.5}$ components in urban environments,[59] 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.~~

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(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 modelling 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 one year before the index date, as diagnosis has been shown previously to occur at a median of 12 months after symptoms onset.[60]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,[61]62 as well as how quickly one is identified as having ALS in the Danish Civil Registration System.[62]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,[63~~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.[~~64~~65] Ultimately, we were limited by what was available in the Danish Civil Registration System.[~~62~~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).[65,66] 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.[65] Matching by finer scale than year of birth was not possible. Bayesian inference allows for full distributional~~

~~estimation of parameters of interest.[66] 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 increase in each of EC, NO_x, CO), and (c) an overall average traffic association (i.e., average percentage change in odds of ALS diagnosis from each of EC, NO_x, CO), while accounting for the variance-covariance structure between the highly-correlated exposures and their coefficients.[66] 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.~~

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

$$logit[\Pr(Y_{ci} = 1)] = \alpha_c +$$

$$\beta_{NO_x}\beta_{EC}\mathrm{EC}_{ci} + \beta_{\mathrm{NO}_x}\mathrm{NO}_{x_{ci}} + \beta_{CO}\mathrm{CO}_{ci} + \beta_{EC}\mathrm{EC}_{ci} +$$

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

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

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); β_{EC}, β_{NO_x}, β_{CO}, β_{EC}, $\beta_{\mathrm{PM}_{2.5}}$ the individual pollutant-specific coefficients (log-odds) per standard deviation (SD) increase in concentration of NO_x, CO, EC, non-EC $PM_{2.5}$ 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 specific 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,[67,68] 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.[68,69] In a sensitivity analysis, we included O_3 in the model, as O_3 concentrations have been associated with many adverse health outcomes,[69,70] 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}, ~~and β_{EC}~~ represent the independent individual pollutant ~~specific~~ associations with ALS diagnosis. In the same model, we estimated the joint association between these three pollutants and ALS diagnosis as:

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

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 ~~specific~~ associations arise from a distribution of the ~~overall~~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:

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

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

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

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

where λ denotes the ~~overall~~ average one-SD association of traffic-related pollution with variance σ_λ. Σ, the estimated variance-covariance matrix among individual pollutant ~~specific~~ estimates, was expressed as a decomposition into a positive-definite correlation matrix Ω and scale matrix τ.[~~70~~71]

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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;[71,72,72,73] Ω was defined by the weakly-informative prior LKJCorr(1).[73,74] 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., ~~$e^{\beta_{\mathrm{NO_2}}} -$~~ $e^{\beta_{\mathrm{EC}}} - 1$, etc. obtained in the modelling process). Due to the risk-set matching pattern of our case-control study, odds ratios are also equivalently incidence ratios (IRs).[65,66] 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[74] ~~was below 1.1 for all estimated model parameters.~~[75] was below 1.1 for all estimated model parameters. The reported 95% credible intervals (CrI) are the 2.5$^{\mathrm{th}}$ to 97.5$^{\mathrm{th}}$ 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[75] and R-STAN, version 2.21.2.[66] 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.~~

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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.

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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 3,939 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 µg/m^3 for EC (SD=0.42 µg/m^3), 27 µg/m^3 for NO_x (SD=20 µg/m^3), and 238 µg/m^3 for CO (SD=106 ~~µg/m^3) and 0.85 µg/m^3 for EC (SD=0.42~~ µg/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~~, EC~~) 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.

For 5-year average pollutant concentrations, we observed the largest overall association for the individual SD increase in EC (11.5%; 95% CrI: -1.0%, 25.6% per 0.42 µg/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 µg/m^3; 27.8% posterior probability of positive association) and CO (-3.2%; 95% CrI: -14.4%, 10.0% per 106 µg/m^3; 26.7% posterior probability of positive association). ~~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 overall traffic association was null (-0.1%; 95% CrI: -17.4%, 20.8%).~~ Non-EC $PM_{2.5}$ was not associated with ALS diagnosis (0.7%; 95% CrI: -9.2%, 12.4%). 1-year EC average exposure was associated with an increase in odds of ALS diagnosis (15.4%; 95% CrI: 1.6%, 25.6~~%) (Figure 2).~~ %). ~~Compared to the 1- and 5-year results, the 10-year average exposure results were attenuated, as associations tended further to the null.~~ Single-pollutant models for each

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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 D) overlapped with the credible intervals of the EC term in the multi-pollutant models (eFigure 1; models B, 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%). 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 1). A map of average concentration of included pollutants (EC, NO_x, ~~EC~~CO, $PM_{2.5}$, ~~CO~~, O_3) across Denmark for a representative year (2000; middle of study period 1989-2013) is also available in eFigure 2.

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Discussion

~~In the largest case-control study of ALS and traffic-related air pollution to date, we found that a joint increase in average concentrations of traffic-related pollutants was potentially associated with an increase in odds of ALS diagnosis, with the clearest results for EC. 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. 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 would have been similar from the single- or multi-pollutant models. The inconsistent associations for NO_x and CO in the multi- and single-pollutant models suggest that the model may have had limited success identifying each individual pollutant's association with ALS diagnosis due to the high level of collinearity of traffic-related pollutants. Nevertheless, the consistency of the sign of the central estimate of EC in all models suggests that EC may be a driver of the ALS and traffic-related pollutant association, though further analysis is required. Our results indicate that traffic-related pollutants, hazardous in many ways,[9–21,40–42] may also be associated with ALS diagnosis. Our finding that increases in EC, are potentially positively associated with ALS diagnosis is plausible. A recent case-control study in the Netherlands reported that ultrafine particles, another traffic emissions related surrogate, were associated with ALS diagnosis,~~In the largest case-control study of ALS and traffic-related air pollution to date, 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. Nevertheless, the consistency of the sign of the central estimate of EC in all models suggests that EC may be a driver of the ALS and traffic-related pollutant association, 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.[76] 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.~~

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[~~77~~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.[61][62] Truck drivers, for whom diesel exposure is common, are also at increased risk of sporadic ALS.[78][79] EC exposure has been associated with inflammation,[79][80] mitochondrial dysfunction[80][81] and DNA damage,[80,81] ~~all of which are plausible pathways of neurodegeneration.~~[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 (0.95 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.[82–84][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.[85][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 used one the largest number 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 ~~joint~~average traffic-related pollutant associations~~, as well as an overall traffic estimate~~. 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, however, are not expected to cause the predicted pollutant concentrations, given exposure assessment. 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 modelled exposure will be inaccurate to some degree. However, any error is not likely correlated with ALS diagnosis, and therefore any bias would be towards null.[~~86~~87] While a previous study found that ALS ascertainment from the Danish National Patient Register was highly reliable,[~~48~~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.[~~87~~88]

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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 (BKMR)[~~88~~89] might be useful in further exploring the robustness of joint associations in a different framework, though BKMR was not appropriate for our particular research question, since BKMR is ~~not~~ currently ~~appropriate~~not available for case-control ~~studies.~~study applications. The timing of exposure will ~~also~~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.

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

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Characteristic	Overall, N = 23,232[a]	Case, N = 3,934[a]	Control, N = 19,298[a]
Average age (years)	66 (12)	66 (12)	66 (12)
Sex			
Female	10,973 (47%)	1,854 (47%)	9,119 (47%)
Male	12,259 (53%)	2,080 (53%)	10,179 (53%)
Socioeconomic status (SES)			
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			
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			
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			
Greater Copenhagen	1,887 (8.1%)	335 (8.5%)	1,552 (8.0%)
Big cities of Denmark	9,385 (40%)	1,590 (40%)	7,795 (40%)
Rest of Denmark	11,954 (51%)	2,008 (51%)	9,946 (52%)
Greenland	6 (<0.1%)	1 (<0.1%)	5 (<0.1%)

[a]Mean (SD); n (%)

693 **Table 2.** Summary of 5-year average pollutant concentrations (all in μg/m^3).

Pollutant	Overall, N = 23,232[a]	Case, N = 3,934[a]	Control, N = 19,298[a]
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)
~~**EC**~~	~~0.85 (0.42)~~	~~0.86 (0.45)~~	~~0.85 (0.42)~~
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)

[a]Mean (SD)

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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 EC, NO_x, CO, and non-EC $PM_{2.5}$ 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.