Abstract

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

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

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

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

Abbreviations:

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

Introduction

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

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

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

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

Methods

Study Population and Outcome Assessment

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

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

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

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

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

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

Exposure data

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

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

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

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

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

Statistical analysis

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

stratum.[66] Matching by finer scale than year of birth was not possible. Bayesian inference allows

for full distributional estimation of parameters of interest.[67] We employed a Bayesian

hierarchical formulation because it enables estimates of (a) independent pollutant–outcome

associations, (b) a joint association of the three pollutants (i.e., total percentage change in odds of

ALS diagnosis with a simultaneous increase in each of EC, NO$_x$, CO), and (c) an average traffic

association (i.e., average percentage change in odds of ALS diagnosis with increases in each of

EC, NO$_x$, CO), while accounting for the variance–covariance structure between the highly

correlated exposures and their coefficients.[67] We included a linear term for each included

pollutant and adjusted for individual- and parish-level SES, civil status, last reported place of

residence, and place of birth.

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

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

$$\beta_{EC}EC_{ci} + \beta_{NO_x}NO_{x_{ci}} + \beta_{CO}CO_{ci} +$$

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

$$\beta_{SES}SES_{ci} + \beta_{Civil\ Status}Civil\ Status_{ci} + \beta_{Residence}Residence_{ci} + \beta_{Birth}Birth_{ci},$$

where Y_{ci} denotes whether subject i in matched stratum c was diagnosed with ALS, i.e., c

represents a case and its matched controls; α_c the matched stratum-specific intercepts (not

estimated in conditional logistic models); $\beta_{EC}, \beta_{NO_x}, \beta_{CO}, \beta_{PM_{2.5}}$ 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

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to scale pollutant concentrations. If other sources of air pollution are associated with ALS, then including non-EC PM2.5 adjusts for PM2.5 from other sources,[68] as well as indicating whether pollution from other sources not explicitly quantified might also have associations with ALS. Therefore, $\beta_{PM_{2.5}}$ 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 PM2.5 concentrations.[69] In a sensitivity analysis, we included O3 in the model, as O3 concentrations have been associated with many adverse health outcomes,[70] and were negatively correlated with traffic-related pollutants, and added $ns(\text{SES}_{\text{parish}_{ci}})$, as a natural spline with three degrees of freedom.

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

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

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

Finally, we assumed that the traffic-related individual pollutant associations arise from a distribution of the average traffic association with ALS diagnosis. We placed a hierarchy on the traffic-specific individual pollutant terms in the model to account for the fact that the traffic-related pollutants, EC, NO$_x$, CO, originate from common sources and primarily traffic in urban environments:

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

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

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

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

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

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

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

converged by checking that the Gelman–Rubin potential scale reduction statistic[75] was below 1.1

for all estimated model parameters. The reported 95% credible intervals (CrI) are the 2.5th to

97.5th percentiles of each parameter's posterior marginal distribution. To calculate the probability

that an association estimate was greater than null, we used the 4,000 samples of the posterior

distribution and took the proportion of samples which were above the null. A 50% probability

means that it is as likely as not that the marginal estimate is null, a probability closer to 100%

indicates that the association is more likely to be truly positive, with closer to 0% indicating

more likely to be truly negative.

We conducted statistical analyses using the R Statistical Software, version 4.1.1[76] and R-STAN,

version 2.21.2.[67] All code for analysis, results from analysis, and visualization presented in this

manuscript is publicly available via GitHub at

https://github.com/rmp15/traffic_air_pollution_als_denmark_epidemiology.

We assessed the sensitivity of our results to hyper-prior assignment; running more iterations and

warm-up per chain; inclusion of O_3; single traffic-related pollutant models adjusting for non-EC

$PM_{2.5}$; as well as adjusting by parish-level SES. From the parish-level SES sensitivity analysis

we excluded those who lived in areas without parish-level SES data, namely: (i) 819 participants

for 1-year average exposure; (ii) 826 participants for 5-year average exposure; and (iii) 838

participants for 10-year average exposure.

Results

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

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

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

associated with a decrease in odds of ALS diagnosis in NO_x (-4.6%; 95% CrI: -18.1%, 8.9% per

20 $\mu g/m^3$; 27.8% posterior probability of positive association) and CO (-3.2%; 95% CrI: -14.4%,

10.0% per 106 $\mu g/m^3$; 26.7% posterior probability of positive association). Non-EC $PM_{2.5}$ was

not associated with ALS diagnosis (0.7%; 95% CrI: -9.2%, 12.4% per 2.37 $\mu g/m^3$; 54.1%

posterior probability of positive association). One-year EC average exposure was associated with

an increase in odds of ALS diagnosis (15.4%; 95% CrI: 1.6%, 25.6% per 0.42 $\mu g/m^3$; 98.9%

posterior probability of positive association). Single-pollutant models for each traffic-related

pollutant adjusting for non-EC $PM_{2.5}$ (eFigure 1; single traffic-related pollutant models D, E and

F) resulted in positive associations for each of EC, NO_x, CO, with positive associations for non-

EC $PM_{2.5}$ in all but the model with EC. The 95% credible interval for EC in the single-pollutant

model (eFigure 1; model F) overlapped with the credible intervals of the EC term in the multi-

pollutant models (eFigure 1; models A to C, G to P). The joint association of traffic-related

pollutants (EC, NO_x, CO) was 2.3% (95% CrI: -3.3%, 7.7%), with an 77.8% posterior

probability of a positive association. The average traffic association was null (-0.1%; 95% CrI: -

17.4%, 20.8%; 45.5% posterior probability of positive association). Compared to the 1- and 5-

year results, the 10-year average exposure results were attenuated, as associations tended further

to the null. Results from variations of the main model in the sensitivity analyses were robust to

prior choices, inclusion of O_3, and inclusion of parish-level SES (eFigure 2).

Discussion

In the largest case–control study of ALS and traffic-related air pollution to date (to our

knowledge), we found that EC had the largest-in-magnitude independent association with ALS

diagnosis, while associations with NO_x and CO were negative with credible intervals

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

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

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

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

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

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

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

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

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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 elemental carbon (EC), nitrogen oxides (NO_x), carbon monoxide (CO), non-elemental carbon fine particles (non-EC $PM_{2.5}$), and ozone (O_3)together, and were additionally adjusted by age, sex, year of birth, vital status, socioeconomic status, civil status, last reported place of residence, and place of birth.