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Original Contribution

Exploring Relevant Time Windows in the Association Between $PM_{2.5}$ Exposure and Amyotrophic Lateral Sclerosis: A Case-Control Study in Denmark

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Studies suggest a link between particulate matter less than or equal to 2.5 μm in diameter ($PM_{2.5}$) and amyotrophic lateral sclerosis (ALS), but to our knowledge critical exposure windows have not been examined. We performed a case-control study in the Danish population spanning the years 1989–2013. Cases were selected from the Danish National Patient Registry based on *International Classification of Diseases* codes. Five controls were randomly selected from the Danish Civil Registry and matched to a case on vital status, age, and sex. $PM_{2.5}$ concentration at residential addresses was assigned using monthly predictions from a dispersion model. We used conditional logistic regression to estimate odds ratios (ORs) and 95% confidence intervals (CIs), adjusting for confounding. We evaluated exposure to averaged $PM_{2.5}$ concentrations 12–24 months, 2–6 years, and 2–11 years pre–ALS diagnosis; annual lagged exposures up to 11 years prediagnosis; and cumulative associations for exposure in lags 1–5 years and 1–10 years prediagnosis, allowing for varying association estimates by year. We identified 3,983 cases and 19,915 controls. Cumulative exposure to $PM_{2.5}$ in the period 2–6 years prediagnosis was associated with ALS (OR = 1.06, 95% CI: 0.99, 1.13). Exposures in the second, third, and fourth years prediagnosis were individually associated with higher odds of ALS (e.g., for lag 1, OR = 1.04, 95% CI: 1.00, 1.08). Exposure to $PM_{2.5}$ within 6 years before diagnosis may represent a critical exposure window for ALS.

air pollution; amyotrophic lateral sclerosis; case-control studies; environmental exposure; exposure windows; fine particulate matter; $PM_{2.5}$

Abbreviations: AIC, Akaike information criterion; ALS, amyotrophic lateral sclerosis; CI, confidence interval; DEHM, Danish Eulerian hemispheric model; GIS, geographic information system; ICD, *International Classification of Diseases*; OR, odds ratio; $PM_{2.5}$, particulate matter less than or equal to 2.5 μm in diameter; SES, socioeconomic status; UBM, urban background model.

Amyotrophic lateral sclerosis (ALS) is a rare and devastating neurodegenerative disease characterized by progressive degeneration of motor neurons. Neuronal degeneration leads to weakening voluntary muscles, resulting in paralysis and eventually death, primarily from respiratory failure (1, 2). Neuronal degeneration develops over the course of years or perhaps decades before clinical symptoms appear (3, 4), but what triggers degeneration or contributes to its progression is still unknown (1, 5). Various genetic variants have been associated with ALS, but only about 5%–10% of cases are familial with a Mendelian inheritance pattern (2, 6). Nearly 90% of ALS cases are classified as sporadic; that is, the patient has no family history of the disease (6). There is also considerable variability in the phenotypic expression of ALS, even among cases that share genetic variants (5, 7). Phenotypic variability can be observed with regard to age at symptom onset, type of motor neuron involvement, nonmotor symptoms, and survival post–clinical diagnosis, among other parameters (3, 5, 7, 8). Altogether, these indicate that the mechanisms contributing to ALS are complex

and likely to involve both genetic and environmental factors (3, 9).

Over the last decade, several experimental studies have shown that fine particulate matter, defined as particulate matter with an aerodynamic diameter less than or equal to 2.5 μm ($PM_{2.5}$), can initiate biological responses that may be of relevance to the pathology of neurodegenerative diseases, including oxidative stress, proteinopathy (10–12), mitochondria damage (13), glutamatergic neurotoxicity (14), and systemic inflammation, in turn linked to neuroinflammation (15, 16). Whether these $PM_{2.5}$-triggered cellular and system-level biological responses contribute to disease remains inconclusive. Epidemiologic studies increasingly suggest a positive link between $PM_{2.5}$ exposure and several neurodegenerative diseases, including dementia (17, 18), but only a few studies have evaluated this association in ALS (19–22).

Although significant advances have been made in our understanding of the genetic contributors to ALS, the role of environmental factors, such as $PM_{2.5}$ exposure, has been more challenging to assess. In contrast to the genome, which is consistent throughout the lifetime, environmental exposures vary over time. Adding to this complexity, environmental exposures may have delayed effects or be adverse only after a cumulative exposure threshold is reached (3). This means that in ALS, which manifests in adulthood and has an unknown latency period, $PM_{2.5}$ exposures that occurred years before overt symptoms appear could potentially be of relevance. However, the rare nature of ALS creates data limitations that restrict statistical power and, as a result, the feasibility of evaluating long-term exposure history in epidemiologic studies.

In this study, we leveraged data from nationwide Danish registries to compile a population-based data set of 3,983 cases and 19,915 controls spanning the years 1989–2013 and $PM_{2.5}$ estimates at the residential address from a prediction model (23) to evaluate time windows of exposure up to 11 years before ALS disease diagnosis. This is one of the most extensive ALS studies of environmental factors carried out to date, and it provides a unique opportunity to evaluate the influence of time on $PM_{2.5}$ exposure. We examined 1-, 5-, and 10-year $PM_{2.5}$ exposure averages preceding the date of the first hospitalization and also assessed both cumulative and delayed associations using a distributed-lag model. Identifying susceptible time windows of exposure to modifiable environmental factors, such as $PM_{2.5}$, can open new avenues to reduce the health and financial burden of ALS and provide insight into potentially critical time points of pathological processes.

METHODS

Study population and data collection

This was a population-based case-control study in the Danish population spanning the period 1989–2013. We obtained patient data from the Danish National Patient Registry, which was established in 1977 and includes nationwide inpatient hospital records for ALS and, as of 1995, outpatient data as well. We identified ALS cases based on their *International Classification of Diseases* (ICD) discharge codes. We used *International Classification of Diseases, Eighth Revision* (ICD-8) code 348.0 (ALS) until 1993 and after that *International Classification of Diseases, Tenth Revision* (ICD-10) code G12.2 (motor neuron disease) and the Danish subcategory code DG12.2G (ALS). We only considered primary diagnosis codes. Given the generally rapid progression of ALS after symptom onset (24), we used the date of the first hospital visit with the relevant ICD code as a proxy for the diagnosis date (the terms "first hospitalization" and "diagnosis date" are used interchangeably hereafter). We included only patients aged 20 years or older at the time of first hospitalization and restricted the analyses to cases identified after 1988 to ensure that we had complete $PM_{2.5}$ exposure histories for at least 11 years before diagnosis. Additionally, given the short survival of ALS patients postdiagnosis (median of 2–3 years) (24, 25), excluding cases from prior to 1989 removed some of the potentially prevalent cases.

We linked patient data from the Danish National Patient Registry to the Danish Civil Registration System via a unique personal identifier number. The Danish Civil Registration System was established in 1968 and includes administrative records (e.g., date, sex, place of birth, vital status, and history of civil status and residential addresses) on all persons living in Denmark—records are kept even after a person dies or emigrates (26).

We selected controls from the Danish Civil Registration System as any persons with no mention of ICD-8 code 348.0 or ICD-10 code G12.2 or Danish National Patient Registry code DG12.2G up to the matched patient's diagnosis date. We matched 5 randomly selected controls to an individual case on age (i.e., same birth year), sex, and vital status (i.e., alive on the date of the case's diagnosis). The matching design has been described in more detail elsewhere (27, 28).

The institutional review boards of Columbia University and the Danish Data Protection Agency approved this study. Participants in our analyses were not required to provide informed consent; by Danish legislation, participants are not required to provide informed consent when no biological samples are obtained.

Covariates

We used the 5-category socioeconomic status (SES) definitions developed by the Danish Institute of Social Sciences, based on job titles obtained primarily from income tax forms. Group 1 refers to the highest status and includes corporate managers and academics; group 2 comprises proprietors, managers of small businesses, and teachers; group 3 includes technicians and nurses; group 4 includes skilled workers (persons who received about 3 years of formal theoretical and practical training (e.g., a mason, carpenter, or hairdresser)); group 5 includes unskilled workers (persons who received no formal training and usually have a lower salary); and group 9 includes unknown and unemployed individuals. If a case/control was married, we used the higher of the couple's individual SES ranks. We also added covariates on civil status (nonmarried, married, divorced, or widowed); place of birth (Greater Copenhagen, other major

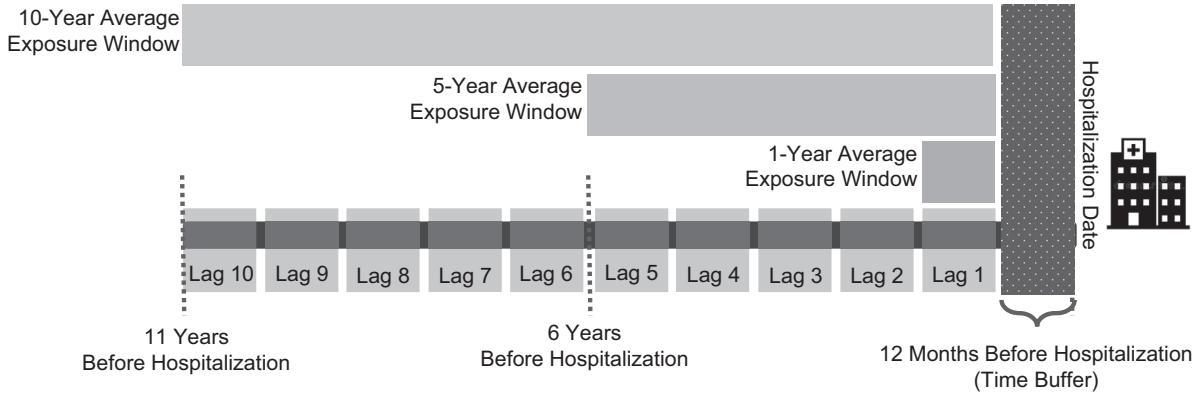

Figure 1. Exposure time windows used in a case-control study of the association between $PM_{2.5}$ exposure and amyotrophic lateral sclerosis, Denmark, 1989–2013. The figure illustrates the time windows analyzed for the average exposures (10 years, 5 years, and 1 year) and the lags included in the distributed-lag analysis (lags of 1–10 years). The cumulative associations for exposure to lags 1–5 and 1–10 were also estimated. The dotted area on the right depicts the 12 months before hospitalization, which was excluded from all exposure windows. $PM_{2.5}$, particulate matter less than or equal to 2.5 μm in diameter.

cities, the rest of Denmark, Greenland, a foreign country, or unknown), to adjust for location-specific early-life potential confounders; and place of residence on the date of the first hospitalization (capital region of Denmark, cities with populations greater than 50,000, Greenland, or the rest of Denmark), to adjust for concurrent exposures that could be associated with air pollution and ALS ascertainment. Lastly, to improve SES characterization, we included a variable on neighborhood-level educational attainment (percentage of the population with a high school diploma or higher).

We obtained neighborhood-level SES data (educational attainment) from Statistics Denmark (29) for 2009 at the parish level. We assigned neighborhood-level SES to cases and controls based on residence on the diagnosis date, which may or may not have been in 2009. This resulted in several participants (3.6%) missing parish-level SES data, because some Danish parishes changed over time (e.g., merged into new parishes or split from 1 parish into 2 or more).

Exposure assessment

We obtained all historical addresses of cases and controls from the Danish Civil Registration System from January 1, 1979, to the diagnosis date, including the dates of moving to and leaving an address. We extracted the geographic coordinates at the house's door of each residence in the residential history of the participants and used this information to assign $PM_{2.5}$ concentrations.

We used $PM_{2.5}$ predictions (in $\mu g/m^3$) obtained from a spatiotemporal model (30) that has been used in previous epidemiologic studies in Denmark (31, 32). The model estimates $PM_{2.5}$ concentrations using a multiscale dispersion modeling system, DEHM/UBM/AirGIS (30). Based on the Danish Eulerian hemispheric model (DEHM), the urban background model (UBM), and a geographic information system (GIS), DEHM/UBM/AirGIS is a highly spatiotemporally resolved human exposure modeling system that integrates air pollution dispersion models, digital maps, national and local administrative databases, concentrations of air pollutants at the regional, urban background, and street levels, meteorological data, and a GIS. Specifically, DEHM/UBM/AirGIS predicts air pollution concentrations at each residential address as the sum of 3 contributions: 1) local air pollution from street traffic, calculated from the intensity and type of traffic, emission factors for the vehicle fleet, street and building geometry, and meteorology (33); 2) urban background concentrations calculated via the UBM (34) using a local scale model based on a high-resolution emission database at a 1 km × 1 km resolution that covers all of Denmark; and 3) regional background concentrations, using a regional chemical transport model, the DEHM (35), covering Denmark (5.6 km × 5.6 km), Northern Europe, and the whole Northern Hemisphere. The final product is highly spatially resolved $PM_{2.5}$ predictions with an average predictive accuracy of $R = 0.83$. In general, underlying uncertainties in the model are slightly higher for earlier years as compared with more recent years, and the model performs similarly in urban and rural sites (30). We estimated monthly $PM_{2.5}$ exposure at the address level for each participant. Then, we averaged the monthly estimates into various exposure windows as described below.

Time windows of exposure

The time windows of exposure are illustrated in Figure 1. All exposure windows excluded the 12 months preceding the date on which patient was first hospitalized and given an ALS ICD code for the first time. These 12 months provided a time buffer for the likely delay between diagnosis and the first hospitalization (36, 37). We evaluated 3 time windows that captured averaged $PM_{2.5}$ concentrations in the 13–24 months, 2–6 years, and 2–11 years preceding the first hospitalization (hereafter referred to as 1-, 5-, and 10-year averaged exposures). Additionally, to evaluate potential

delayed and cumulative associations from persisting $PM_{2.5}$ exposures over time, we analyzed yearly exposure lags back to 11 years (lags 1–10) prediagnosis and estimated cumulative associations in the lag periods 1–5 years and 1–10 years. The lag analysis excluded lag 0, corresponding to the 12 months preceding hospitalization. The averaged association estimates differ from the cumulative estimates in that the former assume equally distributed risk over time while the latter allow estimates to vary across years.

We set an inclusion criterion for each exposure window (see Web Figure 1, available at https://doi.org/10.1093/aje/kwad099). For the 1-year time window and lag analysis, we included only cases and controls with exposure estimates for at least 9 out of 12 months and at least one month measurement for each season (spring, summer, fall, and winter). For the 5- and 10-year time windows, we only included participants with at least 50% of exposure estimates (30 months and 60 months, respectively).

Statistical analysis

We used ALS as the binary outcome in a conditional logistic regression analysis, which accounts for the case-control matching factors (age, sex, and vital status), to estimate odds ratios (ORs) and their corresponding 95% confidence intervals (CIs) per 1-$\mu g/m^3$ increase in $PM_{2.5}$ concentration. We fitted separate models for the 1-, 5-, and 10-year averaged exposures. For the lag analysis, we applied conditional logistic regression within the distributed-lag modeling framework (38). Distributed-lag modeling simultaneously models the shape of the exposure-response relationship within lag and across lags (i.e., lag-response relationship) (38). We modeled the exposure-response relationship linearly and allowed the coefficient on each lag to vary across years in a smooth (nonlinear) way. We explored lag constraints of 3 and 4 degrees of freedom (df) in the lag response and used the Akaike information criterion (AIC) to select the best-fitting model. From the distributed-lag model, we extracted lag-specific and cumulative associations for lag periods 1–5 and 1–10 years and the corresponding 95% CIs. All of the models adjusted for potential socioeconomic and geographic confounding through the inclusion of civil status, place of birth, residence, and occupational category.

Sensitivity analysis

We tested for deviations from linearity in the exposure-response relationship in the 5- and 10-year averaged exposure models using 3-df natural splines and compared model fitness (i.e., linear vs. nonlinear) using the AIC. We also ran all models from the main analysis without adjusting for parish-level SES to evaluate the robustness of our results to neighborhood-level SES adjustment. In addition, we ran all models from the main analysis without adjusting for parish-level SES and including the individuals with missing parish-level SES.

We tested for effect modification by sex (male vs. female), age (>65 years vs. ≤ 65 years), and year of diagnosis (1989–1999 vs. 2000–2013). We used separate distributed-lag models for each of the categories. We modeled the exposure-response relationship linearly and allowed the coefficient on each lag to vary across years in a smooth (nonlinear) way using a constraint of 3 df. We extracted the lag-specific estimates from each model and the cumulative estimates in lags 1–5 and 1–10 (OR and corresponding 95% CI). All models adjusted for potential socioeconomic and geographic confounding by including the same variables as those in the main analysis.

Lastly, we performed a sensitivity analysis to evaluate potential outcome misclassification resulting from the inclusion of outpatient hospitalizations. This model had the same structure as that in the main analysis (e.g., exposure-response relationship modeled linearly, 3 df in the lag constraint, confounder adjustment), but the analysis was restricted to only inpatient data.

RESULTS

Descriptive analysis

We identified 3,983 cases (438 persons diagnosed using ICD-8 code 348.0, 3,119 diagnosed using ICD-10 code G12.2, and 426 diagnosed with the subcategory code DG12.2G) and 19,915 controls and found no differences in mean $PM_{2.5}$ exposure level between cases and controls in any window of exposure. Age, sex, and family SES information was available for all cases and controls. A total of 154 (0.6%) participants (23 cases and 131 controls) had missing data on place of birth, which we categorized as "unknown"; 3 controls were missing data on civil status and were removed. Greenland residents (41 (0.2%) in total; 5 cases and 36 controls) were also removed to facilitate model convergence. The parish-level SES variable had the highest amount of missingness, with 827 (3.5%) participants (153 cases and 674 controls) missing this information. Participants with missing parish-level SES data were removed from the main analysis but included in the sensitivity analyses. Lastly, 173 (0.7%) participants (23 cases and 150 controls) did not have residence information and were removed from all analyses.

We also removed cases/controls missing exposure estimates as per the criteria described in the Methods section (Web Figure 1). On average, we had 97.3% of cases and 95.5% of controls retained from the initial total in each exposure window analysis. There was great overlap across the participants included in each exposure window analysis, and the summary demographic and exposure characteristics did not differ. Demographic characteristics of the participants included in the 5-year exposure time window assessment are summarized in Table 1 and are representative of the participants included in the other exposure analyses. The average $PM_{2.5}$ exposure concentrations (across controls and patients) decreased over time, from approximately 16 $\mu g/m^3$ in 1989 to approximately 9 $\mu g/m^3$ in 2013 (Web Figure 2).

Exposure time windows

Averaged exposures. The estimated associations between ALS diagnosis and averaged $PM_{2.5}$ exposure in 1-year (OR = 1.01, 95% CI: 0.98, 1.05), 5-year (OR = 1.00, 95% CI:

Table 1. Characteristics of ALS Cases and Controls Included in the 5-Year Time Window in a Study of the ALS-$PM_{2.5}$ Association, Denmark, 1989–2013[a,b]

Characteristic	Overall (*n* = 23,063)		Cases (*n* = 3,906)		Controls (*n* = 19,157)	
	No.	%	No.	%	No.	%
Sex						
Female	10,876	47.0	1,838	47.0	9,038	47.0
Male	12,187	53.0	2,068	53.0	10,119	53.0
Average age, years[c]	65 (12)		65 (12)		65 (12)	
Civil status						
Married	14,074	61.0	2,400	61.0	11,674	61.0
Divorced	2,685	12.0	427	11.0	2,258	12.0
Widowed	4,188	18.0	722	18.0	3,466	18.0
Never married	2,116	9.2	357	9.1	1,759	9.2
Place of birth						
Greater Copenhagen	4,816	21.0	826	21.0	3,990	21.0
Large city[d] in Denmark	7,866	34.0	1,349	35.0	6,517	34.0
Rest of Denmark	8,951	39.0	1,535	39.0	7,416	39.0
Greenland	239	1.0	52	1.3	187	1.0
Foreign country	1,057	4.6	121	3.1	936	4.9
Unknown	134	0.6	23	0.6	111	0.6
Place of residence						
Greater Copenhagen	7,721	33.0	1,321	34.0	6,400	33.0
Large city[d] in Denmark	3,675	16.0	612	16.0	3,063	16.0
Rest of Denmark	11,667	51.0	1,973	51.0	9,694	51.0
Family SES						
Group 1 (highest)	2,325	10.0	451	12.0	1,874	9.8
Group 2	2,816	12.0	497	13.0	2,319	12.0
Group 3	4,340	19.0	783	20.0	3,557	19.0
Group 4	6,547	28.0	1,066	27.0	5,481	29.0
Group 5 (lowest)	4,385	19.0	709	18.0	3,676	1.0
Group 9 (unknown)	2,650	11.0	400	10.0	2,250	12.0
$PM_{2.5}$ concentration, $\mu g/m^3$[c]	12.62 (2.64)		12.65 (2.68)		12.62 (2.63)	

Abbreviations: ALS, amyotrophic lateral sclerosis; $PM_{2.5}$, particulate matter less than or equal to 2.5 μm in diameter; SES, socioeconomic status.

[a] Participants in the 5-year time window were representative of participants included in the other exposure windows.

[b] The counts presented in this table are slightly lower than the total counts reported in the text because the table presents data only for the cases/controls included in the 5-year exposure window.

[c] Values are expressed as mean (standard deviation).

[d] City with a population greater than 50,000.

0.97, 1.03), and 10-year (OR = 1.00, 95% CI: 0.97, 1.03) lag periods before the first hospitalization were similar in magnitude and null.

Lag and lag-cumulative exposures. We analyzed yearly lag exposures from 11 years before diagnosis to assess potential delayed effects of exposure. Lags were minimally autocorrelated (Web Figure 3). Based on the AIC, the distributed-lag model with 3 df on the lag constraint had a slightly better fit relative to the model with 4 df. The results from these 2 models were similar, and here we present only the results from the better-fitting model (3 df). We found stronger associations with the outcome for exposure time windows closer to the date of diagnosis (lag 1: OR = 1.04 (95% CI: 0.99, 1.08); lag 2: OR = 1.02 (95% CI: 1.01, 1.04); lag 3: OR = 1.00 (95% CI: 0.99, 1.02)). The ORs decreased from lag 4 onward and became null (Figure 2). The lag-cumulative ORs in periods 1–5 and 1–10 years prior to the

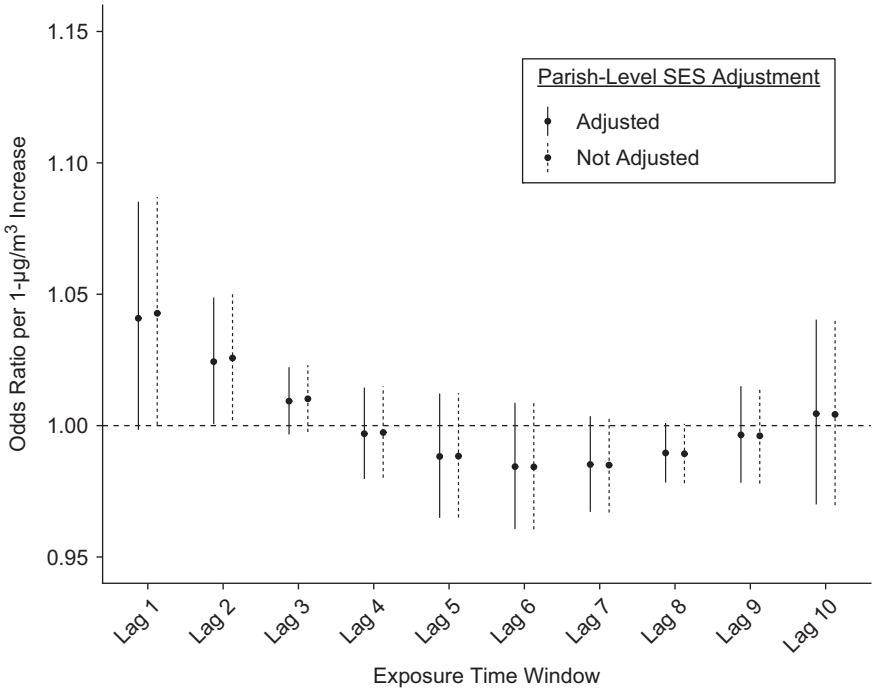

Figure 2. Association between $PM_{2.5}$ exposure and amyotrophic lateral sclerosis (ALS) in a case-control study (distributed-lag model), Denmark, 1989–2013. The odds ratios represent the estimated odds of ALS per 1-$\mu g/m^3$ increase in $PM_{2.5}$ concentration in the given lag; bars represent 95% confidence intervals (CIs). Lag 1 refers to average exposure during the 12–24 months preceding the date of the patient's first hospitalization, lag 2 represents average exposure during the 25–37 months before the first hospitalization, and so on up to 11 years. Results from models that adjusted for parish-level socioeconomic status (SES) are shown with solid 95% CIs, and results from models that did not adjust for parish-level SES are shown with dashed 95% CIs. All models adjusted for individual-level SES, temporal confounding, and geographic confounding using the individual-level covariates civil status, place of birth, place of residence, and occupation. $PM_{2.5}$, particulate matter less than or equal to 2.5 μm in diameter.

first hospitalization were 1.06 (95% CI: 0.99, 1.13) and 1.02 (95% CI: 0.98, 1.05), respectively. Overall, we found a stronger association between 5-year cumulative $PM_{2.5}$ exposure and ALS diagnosis. These results are shown in Figure 3.

Sensitivity analysis. In the sensitivity analyses, we found that adjusting for parish-level SES, in addition to individual-level SES, had a minimal impact on the OR estimates overall (Figures 2 and 3). Our results were also robust to inclusion of the participants with missing data on parish-level SES (Web Figures 4 and 5).

We also conducted a sensitivity analysis to check for potential nonlinearity in the exposure-outcome relationship in the averaged exposure models by adding a natural spline with 3 df on the exposure variable. Based on the AIC, the best-fitting models were models with linear exposure terms.

We detected no differences by sex in the sex-stratified models. The cumulative and lag association estimates for males and females were similar and aligned with the main analysis findings (Web Figure 6). Specifically, we found positive estimates in the lag 1–3 period for both the male and female groups; the cumulative estimates for exposure in the period 1–10 years before the first hospitalization were the same for males (OR = 1.03, 95% CI: 0.99, 1.08) and females (OR = 1.03, 95% CI: 0.99, 1.08), and the cumulative estimates were also similar in the period 1–5 years (males: OR = 1.09 (95% CI: 1.00, 1.19); females: OR = 1.05 (95% CI: 0.96, 1.15)). Nonetheless, we found differences across the age categories (Web Figure 7). The results for patients over 65 years of age reflected the results from the main analyses; we found positive associations between ALS and $PM_{2.5}$ for lag 1–3 and the cumulative exposures. We did not detect an association, however, for patients aged 65 years or younger. We observed the largest difference for lags 1 and 2. We note, however, that the 95% CIs between age groups mostly overlapped.

We also conducted a sensitivity analysis stratified by the year of diagnosis (1989–1999 vs. 2001–2013). In the 1989–1999 group, we found no association between $PM_{2.5}$ exposure and ALS (Web Figure 8). In the 2000–2013 group, we found a positive association between ALS and $PM_{2.5}$ exposure in all lags, with estimates steadily increasing from lag 1–5 and decreasing—but still positive—after lag 6 (Web Figure 8). The cumulative associations were also positive for patients diagnosed during this period, and the 5-year cumulative exposure had the strongest association (OR = 1.16, 95% CI: 1.04, 1.30).

Lastly, we conducted a sensitivity analysis including only inpatient hospitalizations. Overall, the results from that anal-

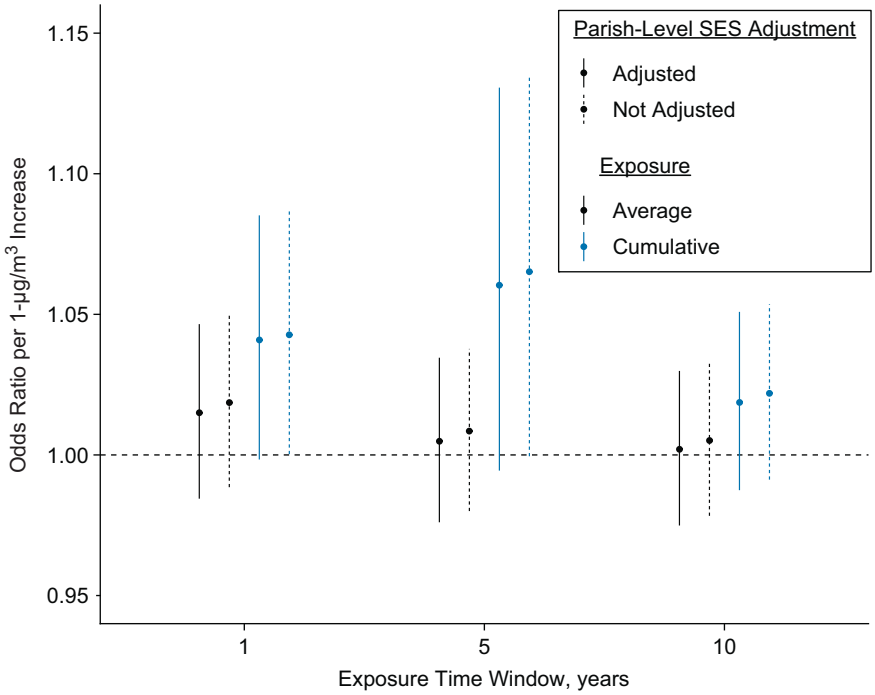

Figure 3. Associations of average and cumulative $PM_{2.5}$ exposures with amyotrophic lateral sclerosis (ALS) in a case-control study, Denmark, 1989–2013. The figure shows the associations between ALS diagnosis and $PM_{2.5}$ exposure 1, 5, and 10 years preceding the date of the first hospitalization; bars represent 95% confidence intervals (CIs). In the averaged exposure models (black points), we used the average $PM_{2.5}$ concentration over each time window as the exposure; thus, the odds ratios represent the estimated odds of ALS per 1-$\mu g/m^3$ increase in the $PM_{2.5}$ average. The odds ratios from the cumulative exposure models (blue points) represent the estimated cumulative association for sustained exposure of each lag over the respective time window—that is, the odds of ALS per 1-$\mu g/m^3$ $PM_{2.5}$ increase in each lag within the exposure window. Results from models that adjusted for parish-level socioeconomic status (SES) are shown with solid 95% CIs, and results from models that did not adjust for parish-level SES are shown with dashed 95% CIs. All models adjusted for individual-level SES, temporal confounding, and geographic confounding using the individual-level covariates civil status, place of birth, place of residence, and occupation. $PM_{2.5}$, particulate matter less than or equal to 2.5 μm in diameter.

ysis aligned with the findings of the main analysis (Web Figure 9).

DISCUSSION

In this study, we compiled one of the largest ALS population-based case-control databases to date by leveraging information from the Danish health and civil registries, and we used a sophisticated GIS-based dispersion modeling system to assign $PM_{2.5}$ exposures to participants at the address level. We evaluated long-term exposure to $PM_{2.5}$ (averaged and cumulative exposures) and yearly exposure lags back to 11 years prediagnosis. Our results suggest that years closer to the disease diagnosis date may be a critical window of exposure to $PM_{2.5}$ in ALS. Specifically, we found that $PM_{2.5}$ exposure during the 6 years preceding diagnosis was associated with ALS risk, but earlier exposures were not.

To our knowledge, only 4 epidemiologic studies have previously evaluated the association between $PM_{2.5}$ exposure and ALS, and only 1 evaluated time windows of exposure. In a county-level analysis in New York State, Nunez et al. (20) evaluated 1-year exposure to predicted $PM_{2.5}$ concentrations associated with ALS disease aggravation and found a positive association. Two Netherlands-based case-control studies evaluated long-term (>1 year) exposure to $PM_{2.5}$ and other air pollutants (19, 22). The authors assigned exposure at the address level by extrapolating and then averaging yearly pollutant estimates prior to the date of diagnosis; the number of years averaged varied across participants. Both of those studies also found a positive $PM_{2.5}$-ALS association. To our knowledge, the current study is the first to have analyzed long-term time windows of exposure. The long-term averaged exposures we estimated were similar to what Seelen et al. (19) and Yu et al. (22) applied, but we estimated monthly exposure concentrations at the address level for each participant rather than extrapolating concentrations from a single year, which should have resulted in better exposure assessment accuracy.

The clinical phase of ALS is easily recognized based on progressive muscle weakness, but the underlying disease process (e.g., at the molecular, cellular, or systemic level) that eventually leads to clinical symptoms almost certainly begins before the clinical phase emerges (4, 39, 40). Broadly, the clinical literature divides ALS into 2 phases: 1) the symptomatic phase, which includes the clinical stages, and

2) the presymptomatic stage, which is divided into the premanifest stage and the prodromal stage (4). The premanifest stage within the presymptomatic stage refers to the early stage with a molecular, cellular, or systemic phenotype but without symptoms of disease; the prodromal stage of the presymptomatic stage includes possible symptoms or signs (e.g., behavioral, emotional, cognitive, and others) that are too subtle to be noticed by most individuals. The symptomatic phase is when diagnosis occurs and is characterized by overt symptoms (4). The length of the presymptomatic stage is uncertain and probably variable among patients, but it is hypothesized to be on the scale of years (4, 39, 40). In this study, our results suggest that $PM_{2.5}$ exposure during the 2–4 years preceding diagnosis is associated with ALS. If this critical window of exposure falls within the prodromal or premanifest stage of ALS, which is likely, we can hypothesize that $PM_{2.5}$ exposure in these stages adds to the ongoing cellular or molecular process of the disease to the point where the body can no longer compensate (39) and thus crosses into the clinical phase.

Inhaled $PM_{2.5}$ is known to reach the lower respiratory system, where it can permeate the lung alveoli and enter the blood circulation (41). The presence of $PM_{2.5}$ in blood circulation damages endothelial and epithelial barriers, including the blood-brain barrier (41, 42). Subsequently, $PM_{2.5}$-induced breakage of the blood-brain barrier can initiate oxidative stress, inflammation, endoplasmic reticulum stress, mitochondrial dysfunction, autophagy, apoptosis, and other damaging processes (41, 43, 44). Thus, a potential mechanism is that molecular and cellular reactions triggered by $PM_{2.5}$-induced breakage of the blood-brain barrier may combine with ongoing cellular processes in the presymptomatic stages (e.g., altered glutamate release, astrocyte activation, altered synaptic circuits (45–47)) to initiate clinical ALS. This hypothesis would also suggest that $PM_{2.5}$ exposure post–clinical diagnosis may contribute to more rapid disease progression and thus shorter survival. Future studies that evaluate long-term exposure to $PM_{2.5}$ in different time windows and its influence on patient survival are necessary to confirm our findings and the contribution of $PM_{2.5}$ exposure to ALS.

The results from the sensitivity analysis suggested that $PM_{2.5}$ exposure is associated with ALS among persons older than 65 years but not among those aged 65 years or younger. Patients diagnosed at a younger age might have a more aggressive phenotype of the disease resulting from genetic risk factors or other environmental exposures that are more substantial contributors to the disease than $PM_{2.5}$ (48); this could mask an association between ALS and $PM_{2.5}$ exposure. These results contradict previous findings from a New York State study (20). Differences in findings, among others, may arise from variations in $PM_{2.5}$ composition. In our analysis, we also found a $PM_{2.5}$-ALS association in patients diagnosed after 2000 but not among those diagnosed in earlier years. Source apportionment studies indicate that sources of $PM_{2.5}$ may be changing (49), which may influence its composition. Thus, differences in association by year of diagnosis may also be due to variations in $PM_{2.5}$ composition, which a previous study suggested may influence its association with ALS (50).

Our study leveraged a large ALS case-control population and well-validated air pollution predictions to explore the $PM_{2.5}$-ALS association at the individual level. We had complete address histories for cases and controls and estimated $PM_{2.5}$ concentrations at the address level up to 11 years prediagnosis. The population-based control selection minimized the risk of selection bias. Our analysis was one of the most comprehensive epidemiologic studies of $PM_{2.5}$ exposure and ALS carried out to date. However, our study still had several limitations. We used predictions of $PM_{2.5}$ exposure at the residential address rather than measurements of personal exposures. This is expected to have resulted in some exposure measurement error. However, differential exposure misclassification related to the outcome is unlikely, and there is no reason to believe that any error would be associated with ALS diagnosis; thus, any resulting bias would likely have been towards the null (51). The model uncertainties in earlier years were higher. This might have led to a larger exposure measurement error for cases diagnosed earlier in our study and their corresponding controls, which could explain the null results in the pre-2000 analysis. We used the first hospitalization as a proxy for disease diagnosis rather than a clinical diagnosis. However, previous studies in the Danish population indicated that hospital discharge data are an accurate proxy for ALS disease diagnosis (52). Additionally, we excluded the 12 months preceding hospitalization as a buffer for the possible delay between diagnosis and the first hospitalization. Other limitations include potential residual confounding by individual-level factors we did not have information about. However, adjustment for personal factors in analyses that use proxy exposure estimates may have a minimal influence on study results (53). Lastly, the chemical composition of $PM_{2.5}$ in Denmark may not be generalizable to other countries, particularly to middle- and lower-income countries where the primary sources of $PM_{2.5}$ are more likely to differ from those in Denmark and concentrations of $PM_{2.5}$ are likely to be higher.

In conclusion, examining time windows of $PM_{2.5}$ exposure in ALS can provide insight into important time points of pathological processes and improve the characterization of the $PM_{2.5}$-ALS association. Our results suggest that the 6 years preceding ALS diagnosis are possibly a critical time window of exposure in the $PM_{2.5}$-ALS association.

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