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Long-Term Air Pollution Exposure and Amyotrophic Lateral Sclerosis in Netherlands: A Population-based Case–control Study

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Background: Recently, there has been increasing evidence that exposure to air pollution is linked to neurodegenerative diseases, but little is known about the association with amyotrophic lateral sclerosis (ALS).

Objectives: We investigated the association between long-term exposure to air pollution and risk of developing ALS.

Methods: A population-based case–control study was conducted in Netherlands from 1 January 2006 to 1 January 2013. Data from 917 ALS patients and 2,662 controls were analyzed. Annual mean air pollution concentrations were assessed by land use regression (LUR) models developed as part of the European Study of Cohorts for Air Pollution Effects (ESCAPE). Exposure estimates included nitrogen oxides (NO_2, NO_x), particulate matter (PM) with diameters of <2.5 μm ($PM_{2.5}$), <10 μm (PM_{10}), between 10 μm and 2.5 μm (PM_{coarse}), and $PM_{2.5}$ absorbance. We performed conditional logistic regression analysis using two different multivariate models (model 1 adjusted for age, gender, education, smoking status, alcohol use, body mass index, and socioeconomic status; model 2 additionally adjusted for urbanization degree).

Results: Risk of ALS was significantly increased for individuals in the upper exposure quartile of $PM_{2.5}$ absorbance [OR = 1.67; 95% confidence interval (CI): 1.27, 2.18], NO_2 (OR = 1.74; 95% CI: 1.32, 2.30), and NO_x concentrations (OR = 1.38; 95% CI: 1.07, 1.77). These results, except for NO_x, remained significant after adjusting additionally for urbanization degree.

Conclusions: Based on a large population-based case–control study, we report evidence for the association between long-term exposure to traffic-related air pollution and increased susceptibility to ALS. Our findings further support the necessity for regulatory public health interventions to combat air pollution levels and provide additional insight into the potential pathophysiology of ALS. https://doi.org/10.1289/EHP1115

Introduction

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease in which motor neuron loss results in paralysis of limbs, speech and swallowing difficulties, and eventually respiratory failure. Fifty percent of patients with ALS die within 3 y of symptom onset (Huisman et al. 2011). The lifetime risk of ALS is 1:300; it can occur at any adult age, with a median age at onset of 63 y (Cronin et al. 2007; Huisman et al. 2011). In addition, 90–95% of ALS cases appear to be sporadic; they are thought to have a complex etiology, most probably caused by an interaction of multiple genetic and exogenous factors (Al-Chalabi and Hardiman 2013). Smoking is thus far the exogenous factor that has been most consistently identified as a risk factor (Armon 2009). Other risk factors remain inconclusive, in part due to study design, lack of replication studies, and relatively small numbers of patients.

Long-term exposure to air pollutants has been linked to increased mortality rates (Beelen et al. 2008, 2014; Cesaroni et al. 2013; Dockery et al. 1993), specifically to cardiovascular diseases (Cesaroni et al. 2014; Raaschou-Nielsen et al. 2012), respiratory diseases (Beelen et al. 2008; Dimakopoulou et al. 2014; Dong et al. 2012), and to a lesser extent to neurodegenerative diseases, including Parkinson's and Alzheimer's diseases (Kioumourtzoglou et al. 2016; Liu et al. 2016; Ritz et al. 2016; Ranft et al. 2009). To date, there has been only one epidemiological investigation, which included 51 ALS cases, into the risk of developing ALS and air pollution using a predominantly hospital-based, case–control design (Malek et al. 2015). This increasing evidence of a possible link between air pollution and neurodegenerative diseases, together with the observation of an association between smoking and the development of ALS, suggests possible involvement of fine particulates in the etiology of ALS. It has been hypothesized that very small (ultrafine) airborne particles are able to cross or impair the blood–brain barrier after systemic translocation, leading to chronic brain inflammation, microglia activation, oxidative stress, and white-matter abnormalities, which are potential biological pathways contributing to ALS (Block et al. 2012; Costa et al. 2014; Levesque et al. 2011).

We investigated the association between multiple air pollutants and the risk of ALS using historic residential data from a large population-based, case–control study on ALS including more than 900 ALS cases and exposure data from the European Study of Cohorts for Air Pollution Effects (ESCAPE) project.

Methods

Study Population

ALS patients, diagnosed between 1 January 2006 and 1 January 2013 in Netherlands, were enrolled into the Prospective ALS

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study in Netherlands (PAN). The PAN is a large population-based case–control study with an estimated capture rate of 81% of all ALS cases in Netherlands (Huisman et al. 2011). All patients newly diagnosed as possible, probable (laboratory supported), or definite ALS, according to the revised El Escorial Criteria, were included (Brooks et al. 2000). Excluded were ALS mimics (e.g., progressive muscular atrophy, primary lateral sclerosis, multifocal motor neuropathy, inclusion body myositis, postpolio syndrome, or cervical myelopathy), and patients who had a first-, second-, or third-degree family member with ALS, defined as familial ALS ($n = 81$). Clinical characteristics, including the date of symptom onset, were extracted from the medical records of all cases.

To ascertain population-based controls, the general practitioner (GP) of the participating patient was asked to select individuals from the patient register in alphabetical order, starting at the surname of the patient and matched for gender and age at symptom onset of the patient (desired case–control ratio 1:2). Controls had to be alive and free of ALS at the date of symptom onset of the corresponding case. In Netherlands, the health-care system ensures that every inhabitant is registered at a GP, which means this list is representative of the population. Spouses or blood relatives of patients were not eligible to be controls to prevent overmatching. The size of the area covered by one GP (who serves on average $\sim$2,000 patients) can be relatively small, especially in urban settings, and the inclusion date of controls can be years apart from the date of symptom onset in cases in the original population matching (because of the time lag between date of diagnosis and date of symptom onset in ALS cases), both of which can affect exposure to air pollution. Therefore, we broke the original match (including 1,117 cases and 2,849 controls) and applied post hoc matching to the cases by gender and age ($\pm$5 y), region of residence (six different regions) and enrollment date ($\pm$1 y). Enrollment date is defined as the date of symptom onset for cases, and the date of inclusion in the study for controls. This definition resulted in a more uniform distribution of enrollment dates between cases ($n = 917$) and controls ($n = 2662$) and broader spatial matching between cases and controls {i.e., average enrollment date for cases was 1 November 2008 [median; interquartile range (IQR) 3 May 2007–1 June 2010] and for controls 23 October 2008 (median; IQR 18 August 2007–22 April 2010)}. Furthermore, there were no statistical differences in demographic characteristics [age, gender, education, area socioeconomic status (SES), and urbanization degree] between the original cohort and the final cohort (after post hoc matching; see Table S1 and Table S2). A diagram of the matching procedure was added to the Supplemental Material (see Figure S1).

The institutional review board of the University Medical Center Utrecht provided ethical approval. All participants gave written informed consent for inclusion in the study.

Exposure Assessment

We estimated long-term exposure to air pollutants at the residential address of the study participants from 1992 to the date of enrollment in the study using recently developed land use regression (LUR) models in the ESCAPE Project (Beelen et al. 2013; Eeftens et al. 2012). In brief, air pollution was repeatedly measured at multiple locations in 2009 to derive average annual concentration of NO_2, NO_x (nitrogen oxides), and $PM_{2.5}$ (particulate matter (PM) with diameters of < 2.5 μm), PM_{10} (diameters < 10 μm), PM_{coarse} (fraction of PM calculated as the concentration of PM_{10} minus that of $PM_{2.5}$), $PM_{2.5}$ absorbance (marker for soot or black carbon). Subsequently, LUR models were developed to explain the spatial variation in air pollutants by variables such as road networks, traffic intensity, population density, and land use. Cross-validation R^2 to evaluate model performance in Netherlands for different pollutants were $PM_{2.5}$ 61%; $PM_{2.5}$ absorbance 89%; PM_{coarse} 38%; and NO_2 80%. These LUR models were then used to estimate annual ambient air pollution concentration at the participants' addresses. To allow for variation in air pollution concentrations over time, as case–control recruitment varied between 2006 and 2013, we extrapolated modeled concentrations in 2009 back in time to 1992, the earliest year for which routine monitoring information on air pollutants is available in Netherlands (Beelen et al. 2014). In short, predicted concentrations were extrapolated back in time using the absolute difference and the ratio between the baseline year and 2009, based on data from routine background monitoring network sites. Constant concentrations were assumed for the period 2009–2013. Subsequently, we averaged the average annual air pollutant concentrations for each individual from 1992 to the date of onset or inclusion in the study. If more than 50% of their addresses were missing between 1992 until onset or inclusion, participants (22 cases, 15 controls) were excluded. In sensitivity analyses, we also performed analyses without any historical back extrapolation (i.e., air concentrations as predicted in 2009) or by using the air pollution estimates for 1992 for the whole population.

Statistical Analysis

Average annual air pollution exposure was divided into quartiles, based on the exposure distribution among the controls. Conditional logistic regression models were used to determine the association between exposure to air pollutants and ALS. In addition, *p*-values for linear trends were calculated using the median value in each quartile as a continuous variable. We specified two *a priori* models to adjust for confounding based on known and suspected risk factors of ALS. Data on confounder variables [education, body mass index (BMI), smoking status, and alcohol use] were available from questionnaires used in the PAN study; SES and urbanization degree were based on the area level at the participants' addresses. Model 1 was adjusted for age, gender, education (three levels: elementary school, middle/high school, and college/university), and BMI (premorbid, meaning before symptom onset for cases, and at inclusion for controls); current (before symptom onset in cases, and at inclusion for controls) smoking status (as previously found to be the strongest predictor of ALS risk in this population) (De Jong et al. 2012); current alcohol use (before symptom onset for cases, and at inclusion for controls); and area SES (percentage high income at the municipality level of residency). In model 2 we added urbanization degree as a potential confounder to allow for a superior control on urban, peri-urban and rural differences in lifestyle and other environmental factors. Urbanization degree was defined based on the categories of Statistics Netherlands, with an ordinal variable representing very highly urbanized areas >2,500 addresses per square kilometer; highly urbanized 1,500–2,500 addresses per square kilometer; moderately urbanized 1,000–1,500 addresses per square kilometer; low urbanized 500–1,000 addresses per square kilometer; and nonurbanized <500 addresses per square kilometer. Missing values of confounder variables were imputed with the R package Hmisc (R Core Team, Vienna, Austria), using multiple reiterations ($n = 10$) of predictive mean matching with optional weighted probability sampling of the other variables.

To disentangle the effects of different pollutants, we included two pollutants simultaneously in the model. We employed these two-pollutant models to $PM_{2.5}$ absorbance, NO_2, NO_x, and $PM_{2.5}$, representing a more traffic-related and less traffic-related pollutant, respectively. A bipollutant model with the more traffic-related

Table 1. Demographic and clinical characteristics of participants.

Characteristics	ALS patients ($n = 917$)	Controls ($n = 2,662$)	p-Value[a]
Male, n (%)	560 (61.1)	1,633 (61.3)	0.88
Age, y, median (IQR)[b]	63.5 (57.0, 70.1)	63.5 (57.5, 69.7)	0.81
Bulbar site of onset, n (%)	321 (35.0)	—	—
El Escorial classification, n (%)			
Definite	166 (18.1)	—	—
Probable	377 (41.1)	—	—
Probable lab supported	215 (23.4)	—	—
Possible	144 (15.7)	—	—
Education, n (%)			
Elementary school	74 (8.1)	190 (7.1)	—
Secondary school/high school	603 (65.8)	1,702 (63.9)	0.23
College/university	240 (26.2)	770 (28.9)	—
Premorbid BMI, kg/m^2, n (%)[c]			
Underweight (<18.5)	30 (3.3)	23 (0.9)	—
Normal weight (18.5 – < 25.0)	512 (55.8)	1,143 (42.9)	<0.001
Overweight (25.0 – < 30.0)	300 (32.7)	1,239 (46.5)	—
Obese (≥30.0)	75 (8.2)	257 (9.7)	—
Current smoking, n (%)[d]	154 (16.8)	346 (13.0)	0.004
Current alcohol consumption, n (%)[d]	699 (76.2)	2,283 (85.8)	<0.001
Area SES, median (IQR)[e]	20.0 (18.0, 23.0)	20.6 (18.0, 24.0)	0.03
Urbanization degree, addresses/km^2, n (%)[f]			
Very high (≥2,500)	121 (13.2)	243 (9.1)	—
High (1,500 – < 2,500)	244 (26.6)	683 (25.7)	—
Moderately (1,000 – < 1,500)	235 (25.6)	659 (24.8)	<0.001
Low (500 – < 1,000)	245 (26.7)	755 (28.4)	—
Very low (<500)	72 (7.9)	322 (12.1)	—

Note: —, data not available; ALS, amyotrophic lateral sclerosis; BMI, body mass index; SES, socioeconomic status.
[a]p-Values were calculated using chi-square test for categorical variables and the Mann-Whitney U-test for continuous variables.
[b]Age at onset in patients, and age at which questionnaire was completed by controls.
[c]Premorbid, meaning before symptom onset in cases, and at inclusion for controls.
[d]Current, meaning before symptom onset in cases and at study inclusion for controls.
[e]Socioeconomic status is based on area level percentage of high income.
[f]Urbanization degree is based on area level, divided into five categories according to Statistics Netherlands (www.cbs.nl).

components (i.e., $PM_{2.5}$ absorbance, NO_2, NO_x) was not possible due to collinearity.

We performed several sensitivity analyses. First, we explored the possible effect of recency of exposure by only counting the last year of exposure or the last 5 y before enrollment. Second, we considered a 1- and 5-y lag prior to study enrollment, to exclude a possible effect of incipient ALS on the association. Third, we restricted the analyses to participants who had not moved during the last year or the last 5 y prior to study enrollment, to exclude reverse causation of patients moving closer to academic treatment centers, which are in the larger cities in Netherlands with higher traffic-related pollution levels. Fourth, we restricted the analysis to the cases and controls with all confounder data available, excluding an effect of imputation. Fifth, we assessed the differences in estimates in conditional versus unconditional logistic regression analyses. Last, we assessed possible effect modification of current smoking status and performed a subgroup analysis according to site of symptom onset (patients with bulbar or spinal onset). Because correction for urbanization degree may lead to overmatching (as the level of air pollution is correlated to urbanization degree), we used model 1 for all sensitivity analyses.

Results

The analyses presented are based on 917 patients with ALS and 2,662 individually matched controls. Clinical characteristics of the patients with ALS, such as age at onset, site of onset, and El Escorial classification, were similar to previously reported patient characteristics in Europe (Table 1) (Logroscino et al. 2010). BMI, current smoking status, current alcohol use, area SES, and urbanization degree differed significantly between patients and controls (Table 1). Data on at least one of the confounder variables—education, BMI, smoking status, and alcohol use—were missing in 27.5% of the participants and subsequently imputed. Sensitivity analysis restricted to the nonimputed population did not result in significantly different results in comparison with the analysis of the total population (see Table S3).

The mean annual concentration for each pollutant is presented in Table 2 according to case–control status. Although differences were small [as in the previous ESCAPE study on all-cause mortality (Beelen et al. 2014)], the mean concentrations were significantly higher among the cases than among controls for PM_{10}, PM_{coarse}, $PM_{2.5}$ absorbance, NO_2, and NO_x ($p < 0.05$, Mann-Whitney U-test). Pearson correlations between the different exposure measures were generally higher than 0.6 (see Table S4).

The odds ratios (ORs) of all air pollutants were elevated among the highest exposed individuals in comparison with the reference category with the lowest exposed individuals for the unadjusted and adjusted models (Table 3). For $PM_{2.5}$ absorbance, NO_2, and NO_x, these ORs were significantly elevated in model 1 [OR = 1.67 (95% CI: 1.27, 2.18), OR = 1.74 (95% CI: 1.32,

Table 2. Mean annual air pollution exposure for ALS patients and controls.

Air pollutants (mean ± SD)	ALS patients ($n = 917$)	Controls ($n = 2662$)	p-Value[a]
PM_{10}, μg/m^3	31.8 ± 1.4	31.6 ± 1.2	<0.001
PM_{coarse}, μg/m^3	10.4 ± 0.7	10.3 ± 0.6	<0.001
$PM_{2.5}$, μg/m^3	21.3 ± 0.9	21.2 ± 0.8	0.08
$PM_{2.5}$ absorbance, 10^{-5}/m	1.43 ± 0.20	1.40 ± 0.18	<0.001
NO_2, μg/m^3	26.9 ± 5.6	26.0 ± 5.2	<0.001
NO_x, μg/m^3	44.7 ± 9.6	43.6 ± 8.9	0.002

Note: Annual air pollution exposure was assessed from 1992 to the date of enrollment in the study, and subsequently averaged for each participant; ALS, amyotrophic lateral sclerosis.
[a]p-Value was calculated using an independent samples t-test.

Table 3. Conditional logistic regression analyses for the association between ALS and exposure to air pollution.

Air pollutants	Unadjusted model[a] OR (95% CI)	Trend *p*-Value	Model 1[b] OR (95% CI)	Trend *p*-Value	Model 2[c] OR (95% CI)	Trend *p*-Value
PM_{10} ($\mu g/m^3$)						
Q1 (≤30.9)	Reference		Reference		Reference	
Q2 (>30.9 – ≤31.6)	0.79 (0.61, 1.02)		0.77 (0.59, 1.00)		0.75 (0.57, 0.98)	
Q3 (>31.6 – ≤32.2)	0.83 (0.63, 1.10)	0.004	0.83 (0.62, 1.10)	0.006	0.77 (0.57, 1.05)	0.19
Q4 (>32.2)	1.29 (0.98, 1.70)		1.29 (0.97, 1.72)		1.12 (0.79, 1.57)	
PM_{coarse} ($\mu g/m^3$)						
Q1 (≤9.9)	Reference		Reference		Reference	
Q2 (>9.9 – ≤10.2)	0.83 (0.65, 1.06)		0.82 (0.64, 1.05)		0.77 (0.60, 1.00)	
Q3 (>10.2 – ≤10.5)	0.97 (0.76, 1.24)	0.003	0.95 (0.74, 1.24)	0.01	0.84 (0.64, 1.11)	0.24
Q4 (>10.5)	1.28 (0.99, 1.64)		1.24 (0.95, 1.61)		1.04 (0.77, 1.41)	
$PM_{2.5}$ ($\mu g/m^3$)						
Q1 (≤20.7)	Reference		Reference		Reference	
Q2 (>20.7 – ≤21.3)	0.99 (0.76, 1.28)		0.98 (0.75, 1.28)		0.92 (0.70, 1.21)	
Q3 (>21.3 – ≤21.7)	0.82 (0.61, 1.11)	0.08	0.85 (0.62, 1.16)	0.10	0.80 (0.59, 1.09)	0.24
Q4 (>21.7)	1.37 (0.99, 1.89)		1.35 (0.97, 1.88)		1.24 (0.89, 1.73)	
$PM_{2.5}$ absorbance ($10^{-5}/m$)						
Q1 (≤1.29)	Reference		Reference		Reference	
Q2 (>1.29 – ≤1.38)	1.13 (0.89, 1.45)		1.14 (0.88, 1.47)		1.11 (0.85, 1.44)	
Q3 (>1.39 – ≤1.49)	1.06 (0.81, 1.37)	<0.001	1.12 (0.86, 1.47)	<0.001	1.09 (0.81, 1.47)	0.002
Q4 (>1.49)	1.65 (1.28, 2.14)		1.67 (1.27, 2.18)		1.57 (1.14, 2.17)	
NO_2 ($\mu g/m^3$)						
Q1 (≤22.5)	Reference		Reference		Reference	
Q2 (>22.5 – ≤25.8)	1.33 (1.05, 1.61)		1.38 (1.09, 1.76)		1.29 (1.01, 1.66)	
Q3 (>25.8 – ≤29.0)	1.17 (0.91, 1.51)	<0.001	1.25 (0.97, 1.63)	<0.001	1.15 (0.85, 1.55)	0.03
Q4 (>29.0)	1.71 (1.32, 2.23)		1.74 (1.32, 2.30)		1.55 (1.08, 2.21)	
NO_x ($\mu g/m^3$)						
Q1 (≤38.2)	Reference		Reference		Reference	
Q2 (>38.2 – ≤42.2)	0.97 (0.77, 1.22)		0.98 (0.78, 1.24)		0.91 (0.71, 1.15)	
Q3 (>42.2 – ≤47.3)	1.07 (0.85, 1.86)	0.001	1.12 (0.87, 1.43)	0.004	0.99 (0.76, 1.30)	0.14
Q4 (>47.3)	1.40 (1.10, 1.78)		1.38 (1.07, 1.77)		1.17 (0.87, 1.57)	

Note: Conditional logistic regression analysis with the exposure divided into quartiles (Q) based on the levels in controls. Three different (multivariate) models are shown.
[a]The unadjusted model shows unadjusted odds ratios (ORs).
[b]Model 1 was adjusted for gender, age, educational level, current smoking status, current alcohol consumption, body mass index (BMI), and area-level socioeconomic status (SES).
[c]Model 2 was adjusted as in model 1, but also for urbanization degree.

2.30), and OR = 1.38 (95% CI: 1.07, 1.77)]. A slight decrease in the association was found between model 1 and the more comprehensive adjustment model 2 (with inclusion of urbanization degree), whereas no difference was observed between the unadjusted model and model 1. In model 2, $PM_{2.5}$ absorbance and NO_2 still showed significantly elevated ORs in the highest exposure category [OR = 1.57 (95% CI: 1.14, 2.17) and OR = 1.55 (95% CI: 1.08, 2.21)]. Additional spline analyses illustrated that the association for each pollutant separately (see Figure S2), showed nonlinear associations for most pollutants with elevated risks more present at higher levels of exposure.

Effect estimates for the more traffic-related components ($PM_{2.5}$ absorbance, NO_2, and NO_x) in the two-pollutant model analyses by adding $PM_{2.5}$ in the same model, did not differ from the estimates in the single-pollutant models (Table 4). Effect estimates for $PM_{2.5}$ in two-pollutant models adjusted for traffic-related components were closer to the null than the estimates in the single-pollutant models (Table 4).

Sensitivity analyses by recency of exposure (see Table S5), by lagging exposures (see Table S6), or by limiting the analyses to people who did not move homes in the last years (see Table S7), did not result in substantially different results. Performing analyses without any historical back extrapolation and using either the predicted 2009 or 1992 exposure levels also produced no marked differences (it did not change any of the significances) in comparison with the original exposure assignment. Unconditional logistic regression analyses showed essentially the same estimates as in the original conditional logistic regression analyses. Stratification by smoking status revealed that the ORs for nonsmokers were similar to the overall ORs, especially for $PM_{2.5}$ absorbance, NO_2, and NO_x (see Table S8). The associations among the current smokers still showed elevated ORs in the highest exposed individuals for $PM_{2.5}$ absorbance and NO_2; they did not, however, show a clear exposure–response relation. The lower number of cases and controls in this stratum can, at least partially, explain this. To further analyze this difference in smoking status, we performed a linear regression analysis using interaction terms between smoking status and the different air pollutants. This analysis showed nonsignificant interactions for PM_{10} ($p = 0.58$), PM_{coarse} ($p = 0.26$), $PM_{2.5}$ ($p = 0.81$), $PM_{2.5}$ absorbance ($p = 0.98$), NO_2 ($p = 0.64$), and NO_x ($p = 0.56$). Patients with a bulbar site of onset showed higher increased ALS risks with exposure to air pollutants in comparison with patients with a spinal onset (see Table S9).

Discussion

In this study, we observed an increased risk of ALS associated with long-term exposure to air pollution, specifically $PM_{2.5}$ absorbance and the nitrogen oxides. Only the association with $PM_{2.5}$ absorbance and NO_2 persisted after adjustment for urbanization degree. Recently, a higher risk of ALS with exposure to hazardous air pollutants was reported, specifically for ambient air aromatic solvents, in a smaller hospital-based study including 51 case–control pairs (Malek et al. 2015). Our observation, obtained in a much larger study with 16 times the number of cases and using a population-based design, adds important new information about the effects of long-term exposure to air pollution and the increased risk of ALS. Also, we were able to include several regulated and common air pollutants, adding to the relevance of the findings for public health.

Table 4. Results from one-pollutant and two-pollutant models for adjusted association between ALS risk and various air pollutants.

Air pollutants	One-pollutant model OR (95% CI)[a]	Two-pollutant model OR (95% CI)[a]
$PM_{2.5}$ absorbance (adjusted for $PM_{2.5}$)		
Q1	Reference	Reference
Q2	1.14 (0.88–1.47)	1.21 (0.92–1.59)
Q3	1.12 (0.86–1.47)	1.20 (0.90–1.62)
Q4	1.67 (1.27–2.18)	1.73 (1.26–2.37)
NO_2		
Q1	Reference	Reference
Q2	1.38 (1.09–1.76)	1.41 (1.11–1.80)
Q3	1.25 (0.97–1.63)	1.28 (0.98–1.67)
Q4	1.74 (1.32–2.30)	1.73 (1.29–2.30)
NO_x		
Q1	Reference	Reference
Q2	0.98 (0.78–1.24)	0.98 (0.77–1.25)
Q3	1.12 (0.87–1.43)	1.13 (0.88–1.46)
Q4	1.38 (1.07–1.77)	1.33 (1.02–1.75)
$PM_{2.5}$ (adjusted for NO_2)		
Q1	Reference	Reference
Q2	0.98 (0.75–1.28)	0.90 (0.69–1.19)
Q3	0.85 (0.62–1.16)	0.75 (0.55–1.04)
Q4	1.35 (0.97–1.88)	1.15 (0.81–1.62)

[a]Main model 1 was used for the comparison; this confounder model was adjusted for gender, age, educational level, current smoking status, current alcohol consumption, body mass index (BMI), and socioeconomic status (SES).

Of the various air pollutants studied here, $PM_{2.5}$ absorbance, NO_2 and NO_x are primary traffic-related pollutants, and therefore have larger spatial concentration differences in urban areas. In contrast, major contributors to inhalable and especially coarse particles (PM_{10}, PM_{coarse}) are secondary road dust, agricultural materials, and construction industries. We specifically found an increased ALS risk associated with the more traffic-related air pollutants $PM_{2.5}$ absorbance, NO_2, and NO_x. Effect estimates for the more traffic-related components (NO_2, NO_x, $PM_{2.5}$ absorbance) in two-pollutant models adjusted for $PM_{2.5}$ did not differ much from the single-pollutant effect estimates, and the risk estimate for $PM_{2.5}$ regressed to the null. These results support the observation that the observed association between air pollution and ALS risk is mostly driven by local traffic-related constituents. Interestingly, diesel exhaust is an important source of traffic-related air pollution, especially in Europe, and this exposure previously has been associated with ALS risk in occupational studies: Elevated ALS risk was reported for truck drivers (Kurtzke and Beebe 1980; Pamphlett and Rikard-Bell 2013), bus drivers (Park et al. 2005), and machine workers and operators (Park et al. 2005; Schulte et al. 1996). Moreover, previous studies have revealed that exposure to airborne particulates in diesel exhaust results in neuroinflammation in the central nervous system, possibly through microglia activation, elevated cytokine expression, and oxidative stress (Gerlofs-Nijland et al. 2010; Levesque et al. 2011). Further toxicological and animal studies have provided evidence for the association with particulate air pollution and the biological pathways leading to neurodegenerative diseases (Block et al. 2012). It has been demonstrated that ultrafine particles can circumvent the blood–brain barrier by deposition on the olfactory mucosa of the nasal region (Lucchini et al. 2012; Tonelli and Postolache 2010). The particles are then translocated along the olfactory nerve into the olfactory bulb of the brain and may travel transneuronally to more distal sites in the brain (Elder et al. 2006). In an autopsy study, residents in highly polluted urban areas had significantly higher concentrations of fine particulates in the olfactory bulb and frontal cortex in comparison with residents in areas with a low level of pollution (Calderón-Garcidueñas et al. 2012). Recent experimental studies showed that there is also another route for small particles to enter the brain: Mice exposed to diesel exhaust had a compromised blood–brain barrier, leading to an increase in neuroinflammatory markers in the brain (Heidari Nejad et al. 2015; Oppenheim et al. 2013). Moreover, histological evidence showed that human and animal brains exposed to high PM concentrations had increased levels of pro-inflammatory cytokines and markers of oxidative stress (e.g., TNF-α, interleukins, NF-κB, Toll-like receptor) (Calderón-Garcidueñas et al. 2012; Elder et al. 2006; Levesque et al. 2011; Peters et al. 2006). Interestingly, these pathological pathways have been suggested not only in Parkinson's and Alzheimer's diseases, but also in ALS (Block et al. 2012; Rodríguez and Mahy 2016).

These potential biological pathways also parallel the observations associated with smoking (Alonso et al. 2010; Armon 2009; Rothstein 2009). As smoking is a known risk factor for ALS, we performed stratified analyses according to smoking status. Results among current nonsmokers were essentially similar to the total population, especially for the traffic-related air pollutants. However, among the current smokers, the effect estimates appeared to be different from those in the total population. This difference can at least partially be explained by the small numbers in some of the strata, leading to imprecise effect estimates; additional analyses looking at the interaction between smoking status and air pollutants did not reveal significant interactions. Overall, the sensitivity analysis indicates that the observed association between air pollution and ALS is not easily explained by residual confounding due to smoking, nor that there is evidence of effect modification by smoking. Interestingly, we did observe a difference in effect estimates for the subgroups of site of symptom onset, with stronger associations for patients with a bulbar onset. This potential association (although with widely overlapping confidence intervals) has as yet not been reported for smoking, and it is not known how exposure to air pollutants may favor a bulbar site of onset. The only speculative reason could be that the bulbar region is physically closer to the olfactory region in comparison with the spinal lower motor neurons. Because ALS seems to spread through the CNS by a prionlike mechanism after the initial trigger, this proximity could explain the more frequent than usual bulbar site of onset (Ravits and La Spada 2009).

The most important limitation of our study is the uncertainty of the air pollution estimates. However, it has previously been reported that LUR models predict the historic spatial variation well, and the ESCAPE models used in this study have been shown to accurately detect known risks of air pollution (Eeftens et al. 2011). Nevertheless, noteworthy limitations in the exposure assessment are that we had data on air pollution exposure only from 1992 onwards. The early years of exposure (before 1992) might also have been relevant in ALS pathogenesis. Previous studies have, however, shown that air pollution assessment from LUR models demonstrate reasonable stability over periods of about 10 y (Eeftens et al. 2011). Ignoring exposures before 1992 may, however, have resulted in some nondifferential misclassification resulting most likely in bias towards the null. We, furthermore, observed that when analyses were corrected for urbanization degree, the observed risks were somewhat lower. This result may indicate either overcorrection (as air pollution is correlated to urbanization degree) or some unmeasured coexposures that correlate to both air pollution and urbanization degree (for example lifestyle factors such as diet, exercise, or stress).

Conclusions

This study provides new clues for pathogenic pathways in ALS, which will ultimately help to improve understanding of the

mechanisms involved and lead to prevention strategies. As it is the first large population-based study to report on this possible association, it is important that the findings are replicated in other population-based studies. The increased risk of developing ALS due to ambient air pollution was observed well below the existing European annual mean limits of 25 $\mu g/m^3$ for $PM_{2.5}$, and 40 $\mu g/m^3$ for PM_{10} and NO_2 (European Parliament and the Council of the European Union 2008). As ambient air pollution levels are modifiable, these data support the necessity for regulatory public health intervention in air pollution exposure levels.

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