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Emma Casey Date

Association between Fine Particulate Matter Exposure and Cerebrospinal Fluid Biomarkers of Alzheimer's Disease among a Cognitively Healthy Population-based Brain Cohort

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# Association between Fine Particulate Matter Exposure and Cerebrospinal Fluid Biomarkers of Alzheimer's Disease among a Cognitively Healthy Population-based Brain Cohort

By

Emma Casey

B.S., DePaul University, 2020
MSPH, Emory University, 2023

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A thesis submitted to the Faculty of the
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## Abstract

Association between Fine Particulate Matter Exposure and Cerebrospinal Fluid Biomarkers of Alzheimer's Disease among a Cognitively Healthy Population-based Brain Cohort

By Emma Casey

**Background:** Epidemiological evidence suggests air pollution adversely affects cognition and increases the risk of Alzheimer's disease (AD), but little is known about the biological effects of fine particulate matter ($PM_{2.5}$) on early predictors of future disease risk.

**Objectives:** We investigated the association between 1, 3, and 5-year exposure to ambient or traffic-related $PM_{2.5}$ and cerebrospinal fluid biomarkers (CSF) of AD.

**Methods:** We conducted a cross-sectional analysis using data from 1,113 cognitively healthy adults (aged 45-75 years) from the Emory Healthy Brain Study (EHBS) in Georgia. CSF biomarker concentrations of $A\beta_{42}$, tTau, and pTau, were collected from participants at enrollment (between 2016-2020) and analyzed with the Roche Elecsys system. Annual ambient and traffic-related residential $PM_{2.5}$ exposures were estimated at a 1 km and 250 m resolution respectively, and 3- and 5-year exposures were computed as averages of the time before specimen collection. Associations between $PM_{2.5}$ and CSF biomarker concentrations in addition to AD positive cut-offs were estimated with multiple linear/logistic regression respectively, controlling for potential confounders (age, sex, race/ethnicity, BMI, and neighborhood socioeconomic status (N-SES)).

**Results:** Interquartile range (IQR; IQR=0.845) increases in 1-year [β: -0.101; 95% confidence interval (CI): -0.18, -0.02] and 3-year [β: -0.078; 95% CI: -0.15, -0.006] residential ambient fine $PM_{2.5}$ exposures were negatively associated with $A\beta_{42}$ CSF concentrations. Associations between ambient $PM_{2.5}$ and $A\beta_{42}$ were similar for 5-year estimates, but not significant (β: -0.076; 95% CI: -0.160, 0.005). AD CSF portfolio positive cut-offs revealed similar and significant associations between ambient $PM_{2.5}$ and $A\beta_{42}$. $PM_{2.5}$ exposures were not associated with tTau, pTau, $tTau/A\beta_{42}$, or $pTau/A\beta_{42}$ levels at enrollment.

**Conclusion:** In our study, $PM_{2.5}$ exposure, was associated with a significant decrease in CSF $A\beta_{42}$ which suggests an increased risk of developing AD. Longitudinal analyses will clarify the effects of $PM_{2.5}$ on AD progression.

# Association between Fine Particulate Matter Exposure and Cerebrospinal Fluid Biomarkers of Alzheimer's Disease among a Cognitively Healthy Population-based Brain Cohort

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# Table of Contents

## Introduction

As life expectancy rises and the U.S. population pyramid continues to age, we are seeing an increase in chronic non-communicable age-related conditions, including Alzheimer's disease and related dementias (AD/ADRD). With the multifactorial nature of AD pathogenesis, building evidence around preventable environmental exposures may ultimately reduce inequities and improve health outcomes. In this regard, accumulating epidemiological evidence demonstrates an association between exposure to air pollution and the prevalence of AD/ADRD. Most of these studies have focused on links between pollutants such as fine particulate matter ($PM_{2.5}$) and cognitive function, incident cognitive impairment, or dementia [1-4]. Incident dementia is often studied using diagnostic codes on insurance billing claims and medical records, facilitating studies with large sample sizes; however, billing data are known to miss true dementia cases [1, 5] and there are no diagnostic codes for the preclinical stages of dementia. Understanding the impact of air pollution on preclinical stages of dementia in older at-risk adults is crucial from a public health perspective, as it will improve the estimation of the burden of disease in association with air pollution by identifying more affected individuals.

Recent systematic reviews highlight the need to expand the scope of current studies on air pollution and dementia risk to include neuropathologically relevant outcomes, such as early indicators of dementia, including AD cerebrospinal fluid (CSF) and plasma-based biomarkers, which can be assessed at the late stage of the preclinical phase[1, 4, 6].A recent study found positive associations between $PM_{2.5}$ and Aβ1-40 from plasma in longitudinal analyses, but no associations were detected between $PM_{2.5}$ andAβ1-42 or the ratio of Aβ1-42/Aβ1-40 [7]. Blood-based biomarkers of protein pathology are less predictive of brain pathology and have shown insignificant changes in Aβ levels as compared to CSF Aβ biomarkers in AD patients [8]. In this light, three clinically validated CSF biomarkers: $Aβ_{42}$, total tau (tTau), and phosphorylated tau (pTau) have been noted as valid proxies for neuropathological changes of AD[6, 9], and specifically, $Aβ_{42}$, has been linked to the abnormal pathologic state of cerebral Aβ in both animal and human models. Since pathological changes related to AD can begin decades before symptoms appear[10], quantifying the relationship between both ambient and traffic-related $PM_{2.5}$ pollution and CSF biomarkers reflective of AD-positive changes will elucidate how exposure influences dementia risk [11].

So far, two epidemiological studies have reported associations between $PM_{2.5}$ and $Aβ_{42}$ in cognitively healthy samples whereas no relationships with tTau or pTau have been noted [12, 13]. However, there are several limitations, including 1) the exposure assessment in Alemany et al. (2021) and Li et al. (2022) which only focused on average 1- or 2-year $PM_{2.5}$ prior to the biomarker assessment, respectively, 2) the relatively small sample size (N=156) in Alemany *et al*. (2021)[13], and 3) the outcome assessment in Li *et al*. (2022)[12] which relied on the Innotest-AMYLOID(1-42) ELISA assay that often shows systematic variability in comparison to the robust Elecsys® β-Amyloid (1-42) CSF assay for AD diagnosis[14, 15].

We aim to characterize the association between long-term ambient and traffic-related $PM_{2.5}$ exposure (1, 3, and 5 years prior to biomarker assessment) and CSF biomarker composition

($A\beta_{42}$, tTau, and pTau, assessed with Elecsys® AD CSF assays) in a dementia-free, aging population, as part of the Emory Healthy Brain Study (EHBS), and test for effect modification by several well-known risk factors for AD/ADRD-related outcomes, including AP*OE*-ε4 status, the strongest genetic risk factor for AD.

## Methods

### *Study Design and Population*

The EHBS is a gerontology-based ongoing research study focusing on the cognitive health of older adults. The EHBS is nested within the Emory Healthy Aging Study (EHAS) and is located around the metro-Atlanta region in Georgia, USA. Our cross-sectional analysis includes data from the baseline visits, which were conducted between 2016 and 2020. The primary aim of the EHBS is to characterize psychological and psychosocial factors associated with normal and abnormal aging through assessment of the central nervous system among EHAS adults 45-75 years old who were free of cognitive impairment in addition to several other chronic conditions (e.g. congestive heart failure, multiple sclerosis, HIV) at enrollment; more details on recruitment and eligibility have been published elsewhere [16].

Demographic characteristics were collected with the online Health History Questionnaire (HHQ) [16]. Individual-level information was self-reported for gender, age, race/ethnicity, educational attainment, and residential address. Participants could choose one or more race(s) from a 5-item list (White/Caucasian, Black/African American, Asian, American Indian/Alaska Native, Hawaiian/Other Pacific Islander). Hispanic ethnicity (Yes/No) was addressed in a separate HHQ question. Data on educational attainment were also self-reported with 7 possible categories ranging from less than high school to professional or doctorate degree. EHBS biannual study visits include neuropsychology tests, biospecimen collection (blood, cerebrospinal fluid, gut microbiome), cardiovascular measures, and retinal/brain imaging. All measures, including anthropometric, are collected by trained clinical research staff for use in the diagnosis and prediction of chronic illness [16].

### *$PM_{2.5}$ Exposure Assessment*

Due to evidence suggesting a relationship between both ambient and, its component, traffic-related $PM_{2.5}$ exposure and cognitive decline and because traffic-related $PM_{2.5}$ is a major exposure source in urban environments like Atlanta, GA[17], we used both sources of $PM_{2.5}$ in our analyses.

We obtained ambient $PM_{2.5}$ exposure data from the publicly available Socioeconomic Data and Application Center (SEDAC) air quality data set for health-related applications [18]. The data set consists of yearly ambient $PM_{2.5}$ levels (in $\mu g/m^3$) estimated at a 1 km spatial resolution using a well-validated ensemble-based prediction model for the contiguous United States (2000-2016). As described by Di *et al*., three machine-learning algorithms, random forest, neural network, and gradient boosting, included a variety of predictor variables from satellite data, land use,

meteorological variables, and chemical transport model simulations to predict $PM_{2.5}$ [19]. The ensemble model then combined these $PM_{2.5}$ predictions with a generalized additive model that allowed for the contribution of each machine-learning algorithm to vary by location [19]. The ensemble model was trained on $PM_{2.5}$ levels measured at 2156 U.S. EPA monitors, validated with 10-fold cross-validation, and produced high-resolution annual $PM_{2.5}$ predictions with an average $R^2$ of 0.89 [19].

Traffic-related $PM_{2.5}$ exposure concentrations (in $\mu g/m^3$) for the metro-Atlanta area had an overall spatial resolution of 200-250 meters from 2002 to 2019. The $PM_{2.5}$ database was generated by two approaches. Briefly, for the period of 2002-2011, the annual $PM_{2.5}$ concentrations were generated by the Research LINE-sources dispersion (R-LINE) model with the calibration according to the actual measures at local ground monitoring site. The details can be found elsewhere. This database had a spatial resolution of 250 meters. For the period of 2012-2019, traffic-related annual $PM_{2.5}$ concentrations were predicted via a land-use random forest model built on training data comprised of the 2015 annual concentrations of traffic-related $PM_{2.5}$ from Atlanta Reginal Commission, road inventory and traffic monitoring data based on measurements from the Georgia Department of Transportation which considered road geometry and traffic volume, land cover data from the National Land Cover Database, and ambient $PM_{2.5}$ data from Atmospheric Composition Analysis (Christensenet al). The random forest model was trained with the R package *randomForest* and 200-250m resolution annual traffic-related $PM_{2.5}$ predictions had an average $R^2$ of 0.80 (Christensen et al). This database had a spatial resolution of 200 meters. Both databases had spatial coverage of approximately 20 counties in GA. Given the reduced coverage of our traffic-related $PM_{2.5}$ exposure estimates compared to the ambient $PM_{2.5}$ data and the geographic spread of our study population, the sample size for these models was n=1,080.

For both ambient and traffic-related exposures, we spatially matched geocoded residential addresses to the closest centroid of grids (based on 1 $km^2$ or 200-250 $m^2$ grids) to assign annual exposures. We then calculated individual 3 and 5-year exposures by averaging yearly predictions prior to specimen collection.

### *AD CSF Biomarker Concentrations*

CSF biospecimens were collected by EHBS research staff via lumbar puncture, CSF collection protocol has been previously described [20]. Amyloid-Beta (1-42) ($A\beta_{42}$), Total-Tau (tTau), and Phospho-Tau (181P) (pTau) CSF levels were analyzed with established assays (INNO-BIA AlzBio3; INNOGENETICS N.V., Gent, Belgium). Briefly, CSF $A\beta_{42}$, tTau, and pTau were quantified using the ElectroChemiLuminescense Immunoassay (ECLIA) Elecsys® AD CSF portfolio on an automated Roche Diagnostics instrument (F. Hoffman-La Roche Ltd). The assays have measuring ranges of 200–1700 pg/mL ($A\beta_{42}$), 80–1300 pg/mL (tTau) and 8–120 pg/mL (pTau). tTau and pTau levels were log-$base_{10}$ transformed for normality in linear models in the statistical analyses. We also examined CSF biomarker ratio outcomes, namely, $tTau/A\beta_{42}$ and $pTau/A\beta_{42}$ which are highly predictive of amyloid positivity based on concordance with amyloid-PET, including for cognitively normal participants [21]. All AD CSF biomarker outcomes

were kept as continuous variables for linear regression analyses and separately dichotomized based on the Elecsys® AD CSF portfolio positive (+) cut-offs for logistic regression analyses ($A\beta_{42}$ ≤1030 pg/mL; tTau >300 pg/mL; pTau >27 pg/mL; tTau/$A\beta_{42}$ >0.28pg/mL; pTau/$A\beta_{42}$ >0.023pg/mL) [6].

### *Covariates*

Sources of confounding were addressed with a directed acyclic graph (DAG; **Figure S1**). Individual-level confounders were conceptualized as factors impacting both residential $PM_{2.5}$ exposure and the outcome measure. Potential confounding factors included in the analysis are sex, age, neighborhood socioeconomic status (N-SES), race/ethnicity, educational attainment, and body mass index (BMI). Due to historic racism and discriminatory land-use practices such as redlining, environmental exposures disproportionately affect low-income and minority populations. For this reason, neighborhood deprivation characteristics were included as confounding variables and effect modifiers as done in our previous work [22]. Race has also been noted as an important factor when interpreting CSF biomarker results [23]. In addition, BMI influences biomarker concentrations and is also related to N-SES through characteristics such as neighborhood walkability, greenspace, and food access [24, 25].

Due to the presence of multi-ancestral groups and small categories in self-reported race/ethnicity, we used a 3-level race/ethnicity variable in the analysis: White/Caucasian, Black/African American, and Other (as summarized in **Table 1**). Similarly, educational attainment was included as a 3-level variable: Master or higher, College, Less than college. Height and weight measurements were used to calculate body mass index (BMI, weight in kilograms divided by height in meters squared) which was used as a continuous variable in all models. N-SES for each participant was established in this study with census-tract level American Community Survey (ACS) defined principal components of neighborhood deprivation (see Li et al.[22] for details) and the Area Deprivation Index (ADI). As described previously in Li et al.[22], three principal components of neighborhood deprivation were calculated based on estimates for 5-year ACS census-tract-level data, including 16 indicators of six socioeconomic domains (poverty/income, racial composition, education, employment, occupation, and housing properties) (**Figure S2, Table S6**) [22]. The ADI is provided in national percentile rankings at the block group level from 1 to 100, where 100 represents the most deprived neighborhood, and was calculated using census block group-level indicators and factor analysis to cluster indicators based on their ability to explain the variance between block groups [26].

### *Statistical Analyses*

We implemented multiple linear and logistic regression models to estimate the relationship between residential $PM_{2.5}$ exposure and AD CSF biomarker levels at enrollment. In these models, biomarker concentrations (linear regression models) or dichotomized AD positive variables (logistic regression models) were assigned as dependent variables in individual regression models and $PM_{2.5}$ estimates along with several confounding variables as independent variables. Since the biomarkers were measured on the same scales, but with

different ranges, we standardized all continuous biomarker measures by converting them to z-scores prior to employing regression analysis to increase comparability of results across different biomarkers. Z-scores were computed for each observation by subtracting the sample mean from each individual value and subsequently dividing by the sample standard deviation. Further, we standardized the $PM_{2.5}$ estimates according to its distribution by dividing the exposure of interest by the IQR of 1-year ambient or traffic-related $PM_{2.5}$ exposure respectively in all models. The general form of the model for all analyses appears below:

$$\text{AD CSF Biomarker Outcome} = \alpha_0 + \beta_1 PM_{2.5} + \gamma_1 Age + \gamma_2 Sex + \gamma_3 Education + \gamma_4 Ethnicity + \gamma_5 BMI + \gamma_6 ADI + \gamma_7 nSES\ PC_1 + \gamma_8 nSES\ PC_2 + \gamma_9 nSES\ PC_3 + \varepsilon$$

Where ε represents the random error term, with an assumed mean of zero and constant variance ~N(0, $\sigma^2$).

### *Effect Modification Analyses*

We tested for effect modification by several well-established risk factors for AD, adding an interaction term between $PM_{2.5}$ and each risk factor in individual regression models. These risk factors included whether a participant had at least one *APOE*-ε4 allele, family history of AD ( indicated by parent or first-sibling diagnosis), gender, age, and ADI. Despite a hypothesized additive genetic effect of the ε4 allele, statistical interaction was assessed dichotomously due to the small number of homozygous ε4 carriers (2.1%; **Table 1**). Family history of AD (no/yes), sex (male/female), and ADI (<50/≥50) were also added as dichotomous variables while interaction with age was assessed continuously. Using the models with interaction, we then tested for effect modification with the *interplot* R package and n=100,000 simulations.

For all statistical analysis, we used R Statistical Software version 4.2.2, and the significance level α=0.05.

## Results

### *Study Population*

After excluding EHBS participants with missing demographic data (n=46), the analytic sample included 1,113 individuals (**Table 1)**. Participants lived primarily around the Atlanta metropolitan area, spanning 489 census blocks in the state of Georgia. The average age of our sample was 61.7 years (SD=6.73), 69.9% were females, and 84.7% identified as White/Caucasian, 10.9% identified as Black/African American, and only 2.9% identified as Hispanic. The average BMI was 25.5 kg/$m^2$ (SD=3.55). Our sample was highly educated with 86.7% having received an associate degree or higher. The ADI was right skewed with a median of 25, indicating half of the sample is in 25% lower deprivation than the national average.

There was spatial variability in ambient $PM_{2.5}$ levels in our study area, with the highest quantile of exposure (10.1-13.21 μg/$m^3$) localized to the south of the city of Atlanta and lowest (5.63-8.98

μg/m$^3$) exposure localized to communities north of Atlanta such as Marietta and Roswell (**Figure 1**, **Table 1**). Traffic-related $PM_{2.5}$ exposure levels had lower concentrations (only estimating the traffic-related component of $PM_{2.5}$) but higher variability with average annual exposures of 1.15 μg/m$^3$ (SD=0.46) compared with average annual ambient exposures of 9.52 μg/m$^3$ (SD=0.76). Traffic-related $PM_{2.5}$ estimates reached a maximum of 5.10 μg/m$^3$ and these levels were localized exclusively to the city of Atlanta. Ambient and traffic-related $PM_{2.5}$ annual exposure concentrations were weakly correlated (Pearson correlation = 0.36). More details on the distribution of and relation between ambient and traffic-related $PM_{2.5}$ exposure concentrations are provided in the supplemental material (**Figures S3 and S4**).

We observed a wide spread of concentrations for CSF $A\beta_{42}$ in the study population (median $A\beta_{42}$ level = 1210, IQR = 692.3). $A\beta_{42}$ concentrations did not show a major departure from normality, but tTau and pTau distributions were skewed (**Figure S5**). After log transformation, Tau concentrations were approximately normally distributed (**Figure S6**). Approximately 36% of participants had $A\beta_{42}$ concentrations less than or equal to 1030 pg/mL which corresponds, on average, to a positive reading for AD as indicated by Elecsys® AD CSF portfolio positive (+) cut-offs. We observed AD positive readings for tTau and pTau cut-offs in 6% of the study population. Based on the pTau/$A\beta_{42}$ ratio AD (+) positive cut-off, we detected amyloid-positivity in 10.6% of participants. Details on the distributions of AD CSF biomarker concentrations and the frequency of biomarker-positivity detected in the sample are provided in **Table 2**.

### *$PM_{2.5}$ and AD CSF biomarkers*

Among the continuous AD CSF biomarkers, higher levels of 1- and 3-year ambient $PM_{2.5}$ exposures were associated with lower $A\beta_{42}$ CSF concentrations at baseline after adjusting for potential confounding variables (**Figure 2A**). Specifically, an IQR (0.845 μg/m$^3$) increase in the 1- or 3-year ambient $PM_{2.5}$ exposure was significantly associated with a -0.09-SD (95% CI: -0.15, -0.02) and -0.07-SD (95% CI: -0.13,-0.005) decrease in $A\beta_{42}$ CSF z-score, respectively, after confounder-adjustment. The associations between 5-year ambient $PM_{2.5}$ (**Figure 2A**) as well as traffic-related $PM_{2.5}$ (**Figure 2F**) exposure estimates and $A\beta_{42}$ CSF were similar, but not significant. No significant associations were detected between ambient (**Figure 2B-E**) or traffic-related (**Figure 2G-J**) $PM_{2.5}$ exposures and tTau, pTau, tTau/$A\beta_{42}$, or pTau/$A\beta_{42}$ CSF concentrations at enrollment.

For the AD CSF biomarker positive cut-off outcomes, higher residential ambient $PM_{2.5}$ exposures were associated with increased prevalence of an AD positive (+) $A\beta_{42}$ portfolio reading at baseline with statistically significant associations for an IQR (0.845 μg/m$^3$) increase in 1-year (OR=1.23; 95% CI: 1.09, 1.37), 3-year (OR=1.20; 95% CI: 1.06, 1.33), and 5-year (OR=1.21; 95% CI: 1.07, 1.34) average ambient $PM_{2.5}$ exposure (**Figure 3A**). The associations between traffic-related $PM_{2.5}$ exposures and an AD positive (+) $A\beta_{42}$ portfolio reading at enrollment were similar but not significant (**Figure 3F**). We further observed significant associations between 3- and 5-year traffic-related $PM_{2.5}$ exposures and an AD (+) pTau/$A\beta_{42}$ ratio reading at enrollment, but not for 1-year traffic-related $PM_{2.5}$ exposure (**Figure 3J**) or ambient $PM_{2.5}$ exposure (**Figure 3E**). We did not observe statistically significant associations between ambient (**Figure 3B-D**) or traffic-related (**Figure 3G-I**) $PM_{2.5}$ exposures and AD positive (+) tTau, pTau or tTau/$A\beta_{42}$ portfolio cut-offs.

The associations between ambient $PM_{2.5}$ and $A\beta_{42}$ CSF concentrations and AD positive (+) $A\beta_{42}$ portfolio reading remained significant even after restricting our sample size to only those located in the metro-Atlanta area (**Figure S7, Tables S1, S2,** and **S3**), which is the subsample for which

traffic-related $PM_{2.5}$ exposures estimates were available (sample reduced from $N_{ambient}$=1113 to $N_{traffic}$=1080).

*Effect modification by other common risk factors for AD*

The association of average annual ambient $PM_{2.5}$ exposure and concentrations of $A\beta_{42}$ CSF was not significantly modified by *APOE*-ε4 carriership (p = 0.59), AD family history (p = 0.37), ADI (p = 0.62) or sex (p = 0.67) (**Figure 4A-D**). Similarly, the association of average annual ambient $PM_{2.5}$ exposure and AD positive (+) $A\beta_{42}$ portfolio reading was not significantly modified by *APOE*-ε4 carriership (p = 0.80), AD family history (p = 0.27) , ADI (p = 0.12) or sex (p = 0.72) (**Figure S8A-D**). Effect modification by age was also not statistically significant (p=0.17), but we observed an increasing negative effect of $PM_{2.5}$ on $A\beta_{42}$ CSF levels with increasing age, and significant associations between $PM_{2.5}$ and $A\beta_{42}$ CSF levels starting around 60 years of age (**Figure 4E**); a similar pattern was revealed when looking at the stratified effects of $PM_{2.5}$ on AD positive (+) $A\beta_{42}$ portfolio reading by age (interaction p=0.34) (**Figure S8E**). Results for effect modification analyses appear in (**Tables S4** and **S5**).

## Discussion

In the present study, we examined the impacts of both ambient $PM_{2.5}$ exposure and traffic-related $PM_{2.5}$, a major component of ambient $PM_{2.5}$ in urban environments, on CSF biomarkers of AD in 1,113 cognitively healthy individuals. Our findings show associations between long-term ambient $PM_{2.5}$ concentrations at the residence and decreased $A\beta_{42}$ AD CSF biomarker concentrations (significant for 1- and 3-year average exposures), as well as increased likelihood of an $A\beta_{42}$ AD (+) positive portfolio reading (significant for 1-, 3- and 5-year average exposures). We further found significant associations between 3- and 5-year traffic-related $PM_{2.5}$ exposure estimates a pTau/$A\beta_{42}$ (+) positive portfolio reading at enrollment, but not with ambient $PM_{2.5}$ exposure. We found no associations between ambient or traffic-related $PM_{2.5}$ exposures and pTau or tTau concentrations or their ratios with $A\beta_{42}$. Though not statistically significant, the strength of the association between annual ambient $PM_{2.5}$ exposure and $A\beta_{42}$ AD CSF concentrations differed by age and was particularly pronounced for individuals over the age of 60.

The observed association between $PM_{2.5}$ exposure and the $A\beta_{42}$ AD CSF biomarker as well as pTau/$A\beta_{42}$ ratios, which are equally predictive of amyloid PET status (+/-) as Aβ ratio outcomes[27], among cognitively healthy older adults is consistent with evidence from existing literature. Signs of AD can be detected in the early stages of the AD continuum[15], and decreases in CSF concentrations of $A\beta_{42}$ (a marker of amyloidosis) and elevation in tau species (phosphorylated and total tau) are well-established as pathogenic biomarkers in AD diagnosis [28]. To date, there have been few studies estimating the effects of $PM_{2.5}$ exposure on certified biomarkers of AD in healthy, aging populations One study found a similar relationship between air pollution exposure and $A\beta_{42}$, although they used CSF $A\beta_{42/40}$ ratio to reflect Aβ pathology rather than the individual biomarker measurements[13]. Their estimates were similarly negative, but did not reach significance, likely owing to the relatively small sample size (N=147) [13]. Another study [12]found a statistically significant total effect of ambient $PM_{2.5}$ on $A\beta_{42}$ CSF as well as pTau/$A\beta_{42}$ concentrations among N=1,131 cognitively healthy older individuals, which was further mediated by a CSF biomarker of neuroinflammation, sTREM2 [12].

We found mostly null associations between 1-, 3-, and 5-year average ambient and traffic-related $PM_{2.5}$ exposures and tTau as well as pTau concentrations at enrollment, except for the model with 3-year ambient $PM_{2.5}$ and tTau levels. Likewise, we did not detect any relationship between ambient or traffic-related $PM_{2.5}$ exposures and AD-positivity as indicated by tTau or pTau Elecsys® AD (+) CSF cut-offs. Other studies examining the associations between air pollution and CSF biomarkers of AD in cognitively healthy adults report similar findings, where both Alemany *et al*. (2021) and Li *et al*. (2022) found null associations between $PM_{2.5}$ and pTau as well as tTau CSF concentrations [12, 13].

Stronger associations were detected between ambient $PM_{2.5}$ and AD CSF biomarkers as compared with traffic-related $PM_{2.5}$ exposure. Ambient $PM_{2.5}$ contains emissions from traffic, industry, domestic fuel burning, natural sources including soil dust and sea salt, as well as unspecific sources of human origin [29]. On the other hand, traffic-related $PM_{2.5}$ is a source of ambient $PM_{2.5}$ that includes emissions of organic and inorganic gaseous PM precursors from the combustion of fuels and lubricants [29]. Since both sources contain organic and often toxic particles, we expected to see relationships between both sources of $PM_{2.5}$ and AD CSF biomarkers, and while not significant for $A\beta_{42}$, the associations between traffic-related $PM_{2.5}$ and AD CSF biomarkers were similar to associations with ambient $PM_{2.5}$. More research needs to be done to determine which $PM_{2.5}$ components are particularly harmful to the central nervous system.

While we did not find effect modifications by *APOE*-ε4 carriership or other common risk factors for AD, the association between ambient $PM_{2.5}$ exposure and $A\beta_{42}$ CSF became stronger with increasing age (though not statistically significant). These results could suggest that AD CSF biomarkers might not be sensitive enough to detect AD-related changes in participants < 60 years old, but more research in the population will clarify the most clinically relevant age for biomarker measurement. Previous research suggests that biomarker patterns of $A\beta_{42}$ consistent with stage 1 AD (amyloid pathology only) are first detectable during early middle age (45-54 years), while increases in tTau and pTau are typically not apparent until later (ages ≥ 55 years) [30]. However, this previous study used an unstandardized assay, the INNOTEST ELISA, which often yields systematic variability in comparison to the Elecsys assay. Another potential explanation for the stronger associations among participants older than 60 years could be the higher accumulative $PM_{2.5}$ exposure over the lifetime among older individuals. In line with this hypothesis, one study examining the relationship between $PM_{2.5}$ exposure and AD prevalence found a stronger effect of $PM_{2.5}$ on AD prevalence among those at or above 70 years of age [3].

There are several strengths to be noted, such as, the exposure assessment which included two sources of $PM_{2.5}$, ambient and traffic-related, which were estimated at a high spatial resolution of up to 200m; our outcome assessment which relied on a recommended assay for AD CSF biomarker measurement [14], and for which we observed consistent associations using continuous biomarker concentrations as well as AD positivity cut-offs; our inclusion of several well-known confounders and methods to reduce confounding by neighborhood-level characteristics; and our relatively large sample size (N=1113) of CSF measurements from cognitively healthy older adults free of chronic illness.

In addition to its strengths, this study has several limitations. Given that ADRD progresses over the course of several years or decades, we evaluated the associations with 3- and 5-year average $PM_{2.5}$ concentrations prior to enrollment in addition to the 1-year averages. However, given that exposure was assigned based on the baseline residence and some participants could have relocated in the years prior to the study, the 3- and 5-year estimates are likely to be affected by exposure misclassification. This exposure misclassification is also a potential

explanation for the weaker associations between the 3- and 5-year $PM_{2.5}$ exposures and $A\beta_{42}$ CSF concentrations in comparison to the 1-year exposure concentrations. Our study also only used cross-sectional CSF measurements; longitudinal repeated measures analyses may provide a better understanding of the long-term effect of air pollution on CSF biomarker trajectories of AD. Further, our sample was not representative of the Atlanta metropolitan area, the target population, as it was mainly high SES and white, which limits both the generalizability and transportability of our estimates. Finally, while we looked at two different sources of $PM_{2.5}$, we did not examine the relationship between AD pathology and the components of $PM_{2.5}$. Future studies should consider the components of $PM_{2.5}$ as they are dynamic between ambient and traffic-related sources and could reveal novel relationships between exposure and disease pathogenesis.

In conclusion, our results suggest that exposure to $PM_{2.5}$, even at levels below current primary and secondary standards defined by the Environmental Protection Agency (EPA) for $PM_{2.5}$ (annual average standards with levels of 12.0 $\mu g/m^3$ and 15.0 $\mu g/m^3$, respectively), increases the risk of future AD development. Additionally, our results add to the growing body of evidence which suggests that air pollution directly contributes to neurodegeneration by accelerating $A\beta_{42}$ accumulation in the brain [2, 31].

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## Tables & Figures

**Table 1** Baseline descriptive characteristics for EHBS study participants (≥45 years of age).

| Characteristics[d] | Overall (N=1113) |
|---|---|
| **Age (years)** | |
| Mean (SD) | 61.7 (6.73) |
| Median [Min, Max] | 62.0 [45.0, 77.0] |
| **Sex** | |
| Female | 775 (69.6%) |
| **Body Mass Index (kg/m$^2$)** | |
| Mean (SD) | 25.5 (3.55) |
| Median [Min, Max] | 25.3 [16.8, 38.4] |
| **Race/Ethnicity[a]** | |
| White/Caucasian | 943 (84.7%) |
| Black/African American | 121 (10.9%) |
| Other | 49 (4.4%) |
| **Hispanic** | |
| Yes | 32 (2.9%) |
| **Educational Attainment[b]** | |
| Less than college | 147 (13.2%) |
| College | 478 (42.9%) |
| Master or higher | 488 (43.8%) |
| **Area Deprivation Index[c]** | |
| Mean (SD) | 29.4 (20.1) |
| Median [Min, Max] | 25.0 [1.00, 93.0] |
| ***APOE*-ε4 allele carriership** | |
| No allele | 591 (53.1%) |
| 1 allele | 241 (21.7%) |
| 2 alleles | 23 (2.1%) |
| Missing | 258 (23.2%) |
| **Air pollution concentration** | |
| **1 yr. ambient $PM_{2.5}$ (μg/m$^3$)** | |
| Mean (SD) | 9.52 (0.764) |
| Median [Min, Max] | 9.52 [5.63, 13.2] |
| IQR | 0.845 |

| Characteristics[d] | Overall (N=1113) |
|---|---|
| **3 yr. ambient $PM_{2.5}$ ($\mu g/m^3$)** | |
| Mean (SD) | 9.85 (0.832) |
| Median [Min, Max] | 9.96 [5.99, 12.0] |
| IQR | 1.10 |
| **5 yr. ambient $PM_{2.5}$ ($\mu g/m^3$)** | |
| Mean (SD) | 9.96 (0.735) |
| Median [Min, Max] | 10.1 [6.41, 12.0] |
| IQR | 0.945 |
| **1 yr. traffic $PM_{2.5}$ ($\mu g/m^3$)** | |
| Mean (SD) | 1.15 (0.464) |
| Median [Min, Max] | 1.10 [0.155, 5.06] |
| IQR | 0.523 |
| Missing *n* (%) | 33 (3.0%) |
| **3 yr. traffic $PM_{2.5}$ ($\mu g/m^3$)** | |
| Mean (SD) | 1.26 (0.455) |
| Median [Min, Max] | 1.23 [0.175, 5.56] |
| IQR | 0.479 |
| Missing *n* (%) | 33 (3.0%) |
| **5 yr. traffic $PM_{2.5}$ ($\mu g/m^3$)** | |
| Mean (SD) | 1.25 (0.432) |
| Median [Min, Max] | 1.21 [0.168, 5.38] |
| IQR | 0.447 |
| Missing *n* (%) | 33 (3.0%) |

[a]Self-reported race/ethnicity was categorized into 3 groups: White/Caucasian, Black/African American, and Other which includes both minority and mixed ancestry groups.
[b]Educational attainment was self-report and included less than high school, high school/GED, some college, associate's degree, bachelor's degree, master's degree, and a professional degree (e.g., Ph.D.).
[c]Derived from factor analysis and validated to the Census Block Group neighborhood level with factors for the theoretical domains of income, education, employment, and housing quality; higher scores indicate higher levels of "disadvantage."
[d]Abbreviations: yr., Year; SD, standard deviation; Min, minimum; Max, maximum; IQR, interquartile range; µg, Microgram; $m^3$, meters cubed.

**Table 2** Baseline AD CSF Outcomes at enrollment between 2016 and 2020.

| AD CSF concentrations and (+) cut-off | Total (N=1113) |
|---|---|
| **$A\beta_{42}$ (pg/mL)** | |
| Mean (SD) | 1200 (382) |
| Median [Min, Max] | 1210 [200, 1700] |
| IQR | 692.3 |
| **tTau (pg/mL)** | |
| Mean (SD) | 187 (70.9) |
| Median [Min, Max] | 174 [80.0, 799] |
| IQR | 79.6 |
| **pTau (pg/mL)** | |
| Mean (SD) | 16.7 (7.16) |
| Median [Min, Max] | 15.2 [8.00, 83.8] |
| IQR | 7.63 |
| **tTau/$A\beta_{42}$ (pg/mL)** | |
| Mean (SD) | 0.171 (0.107) |
| Median [Min, Max] | 0.141 [0.0818, 1.72] |
| **pTau/$A\beta_{42}$ (pg/mL)** | |
| Mean (SD) | 0.0154 (0.0116) |
| Median [Min, Max] | 0.0123 [0.00692, 0.200] |
| **$A\beta_{42}$** | |
| (+) | 402 (36.1%) |
| **tTau** | |
| (+) | 68 (6.1%) |
| **pTau** | |
| (+) | 65 (5.8%) |
| **tTau/$A\beta_{42}$** | |
| (+) | 87 (7.8%) |
| **pTau/$A\beta_{42}$** | |
| (+) | 118 (10.6%) |

[a]AD CSF portfolio positive (+) vs (-) cut-offs ($A\beta_{42}$ ≤1030 pg/ml; tTau >300 pg/mL; pTau >27 pg/mL; tTau/$A\beta_{42}$ >0.28pg/mL; pTau/$A\beta_{42}$ >0.023pg/mL)

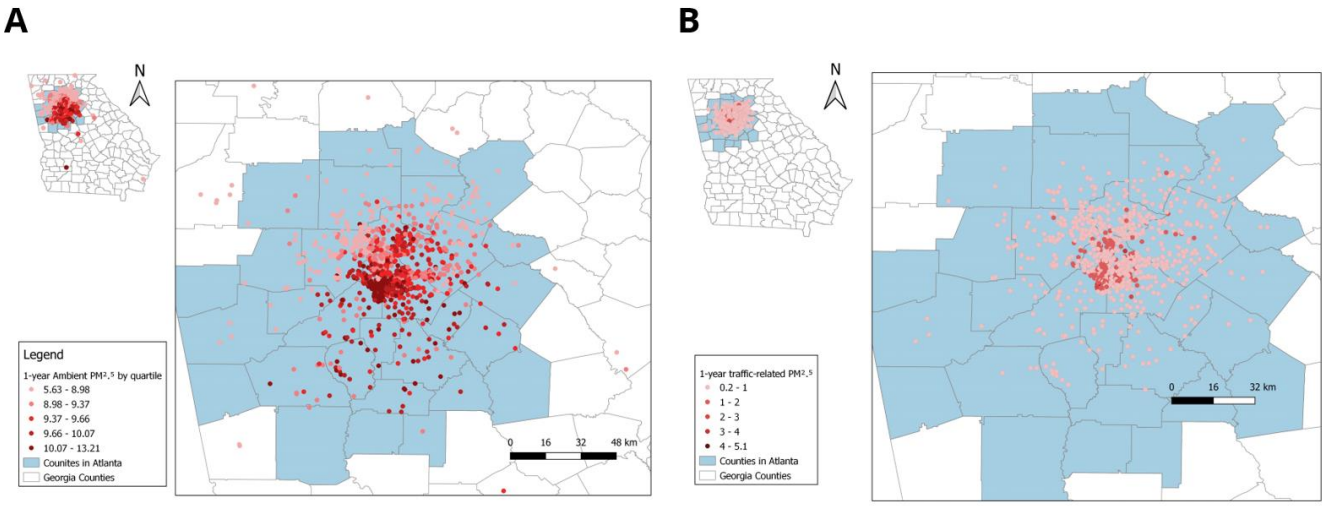


**Figure 1** Map of the geographic distribution, each dot representing a study participant, of our study population and annual ambient residential $PM_{2.5}$ (in $\mu g/m^3$) exposure by quantile (**A**) (N=1113) or annual traffic-related residential $PM_{2.5}$ exposure (min-max) with easy breaks (**B**) (N=1080).

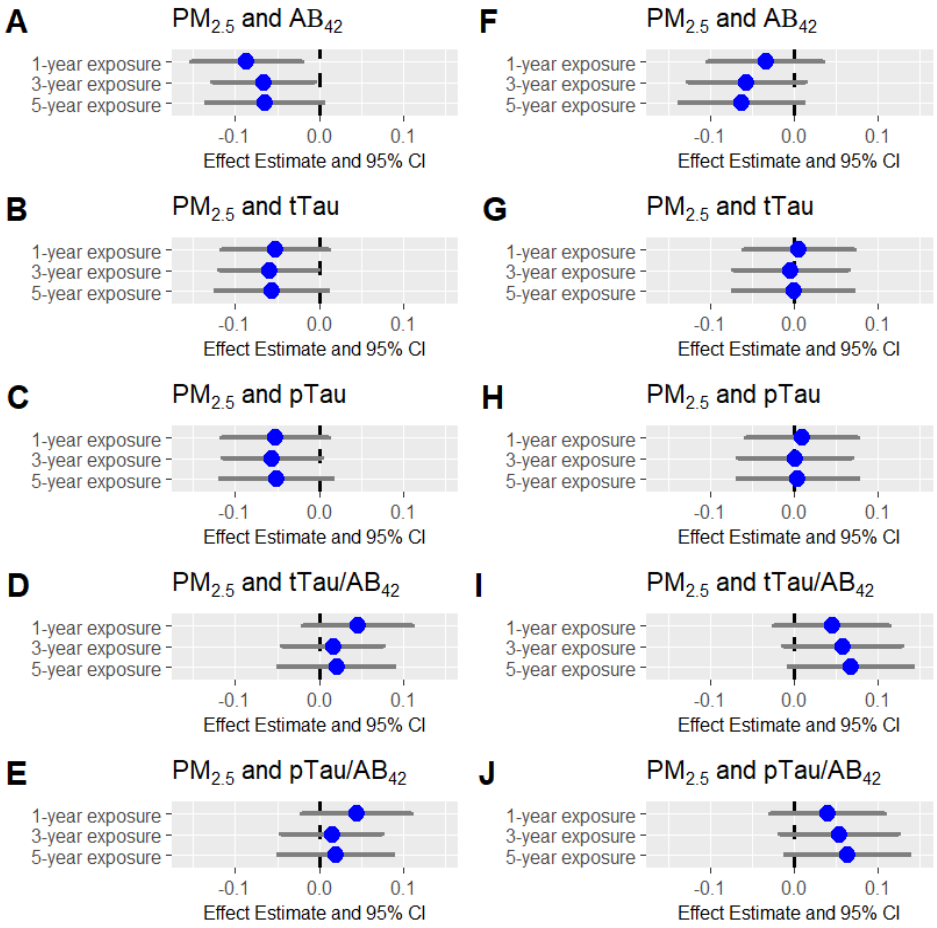


**Figure 2** Effect Estimate (± 95% CI) of 1, 3, and 5-year **A**–**E** (N=1113) ambient and **F**–**J** (N=1080) traffic-related $PM_{2.5}$ exposure on AD CSF Biomarker concentrations (in pg/mL) ($A\beta_{42}$, tTau, pTau, tTau/$A\beta_{42}$, and pTau/$A\beta_{42}$). All estimates are standardized and adjusted for sex, age, N-SES, ethnicity, educational attainment, and BMI. The dashed line indicates the significance threshold: 0 for linear regression.

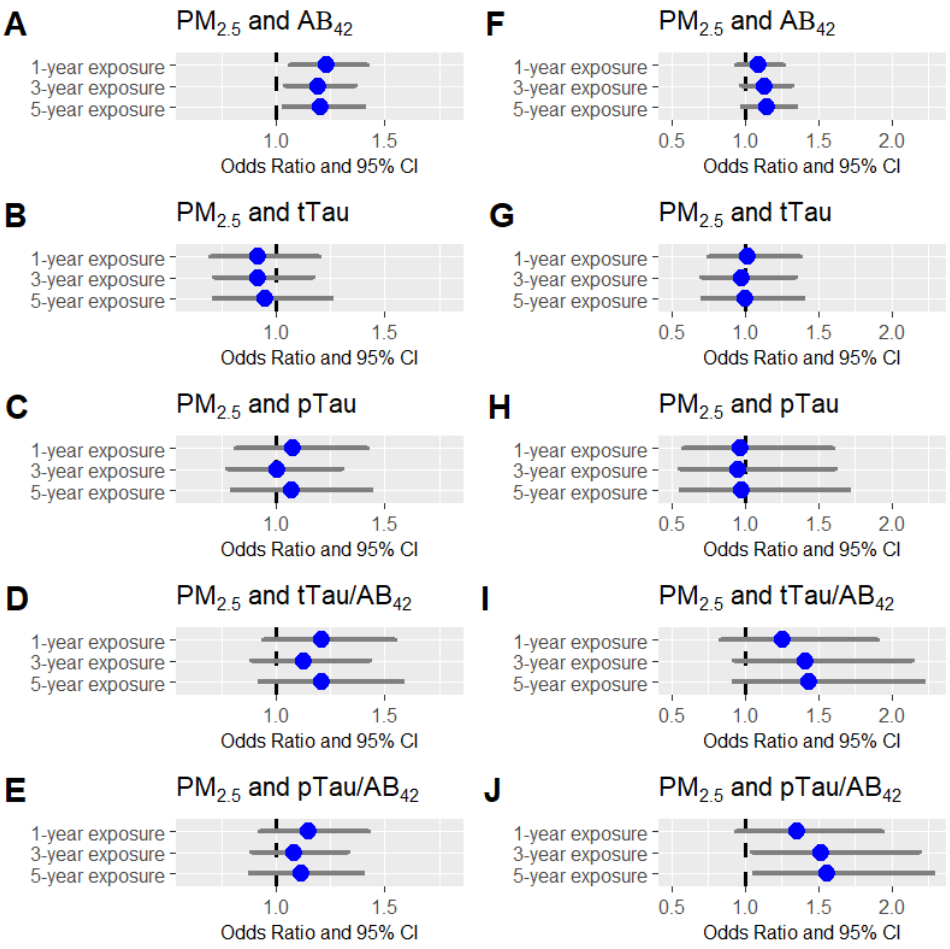


**Figure 3** Odds ratio (± 95% CI) for the association between 1, 3, and 5-year **A**–**E** (N=1113) ambient and **F**–**J** (N=1080) traffic-related $PM_{2.5}$ exposure and AD CSF Biomarkers positive (+) cut-offs ($A\beta_{42}$, tTau, pTau, tTau/$A\beta_{42}$, and pTau/$A\beta_{42}$). All estimates are adjusted for sex, age, N-SES, ethnicity, educational attainment, and BMI. The dashed line indicates the significance threshold: 1 for logistic regression.

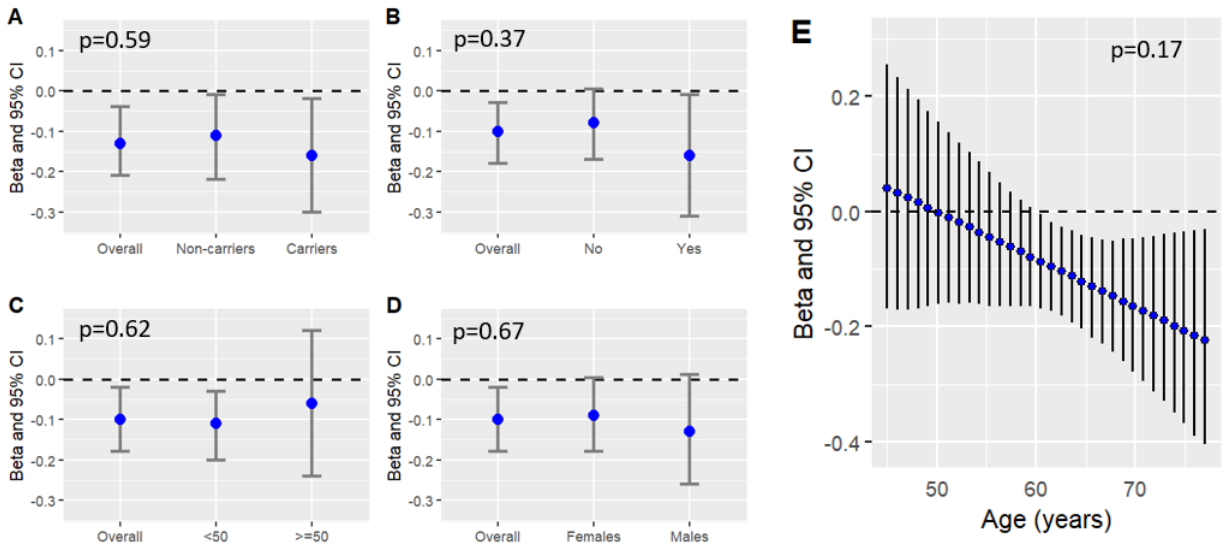


**Figure 4** Effect (± 95% CI) of yearly ambient $PM_{2.5}$ exposure on $A\beta_{42}$ CSF concentrations (in pg/mL) by *APOE*-ε4 carriership (**A**), AD family history (**B**), ADI (**C**), Sex (**D**), and Age (**E**). Presented as overall and stratified effects for dichotomous variables and as continuous for Age, with interaction p-values depicted on each graph. The dashed line indicates the significance threshold: 0 for linear regression. The overall effect in **Figure 4A** (N=855) differs slightly from **Figure 4B-D** (N=1113) due to the decreased sample size after including only participants with *APOE* genotype data.

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