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Fine Particulate Matter and Markers of Alzheimer's Disease Neuropathology at Autopsy in a Community-Based Cohort

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Abstract.
Background: Evidence links fine particulate matter ($PM_{2.5}$) to Alzheimer's disease (AD), but no community-based prospective cohort studies in older adults have evaluated the association between long-term exposure to $PM_{2.5}$ and markers of AD neuropathology at autopsy.
Objective: Using a well-established autopsy cohort and new spatiotemporal predictions of air pollution, we evaluated associations of 10-year $PM_{2.5}$ exposure prior to death with Braak stage, Consortium to Establish a Registry for AD (CERAD) score, and combined AD neuropathologic change (ABC score).
Methods: We used autopsy specimens (N = 832) from the Adult Changes in Thought (ACT) study, with enrollment ongoing since 1994. We assigned long-term exposure at residential address based on two-week average concentrations from a newly developed spatiotemporal model. To account for potential selection bias, we conducted inverse probability weighting. Adjusting for covariates with tiered models, we performed ordinal regression for Braak and CERAD and logistic regression for dichotomized ABC score.
Results: 10-year average (SD) $PM_{2.5}$ from death across the autopsy cohort was 8.2 (1.9) $\mu g/m^3$. Average age (SD) at death was 89 (7) years. Each 1 $\mu g/m^3$ increase in 10-year average $PM_{2.5}$ prior to death was associated with a suggestive increase in the odds of worse neuropathology as indicated by CERAD score (OR: 1.35 (0.90, 1.90)) but a suggestive decreased odds of neuropathology as defined by the ABC score (OR: 0.79 (0.49, 1.19)). There was no association with Braak stage (OR: 0.99 (0.64, 1.47)).

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Conclusion: We report inconclusive associations between $PM_{2.5}$ and AD neuropathology at autopsy among a cohort where 94% of individuals experienced 10-year exposures below the current EPA standard. Prior studies of AD risk factors and AD neuropathology are similarly inconclusive, suggesting alternative mechanistic pathways for disease or residual confounding.

Keywords: Air pollution, Alzheimer's disease, autopsy, dementia, neuropathology, particulate matter

INTRODUCTION

Neurodegenerative diseases, including Alzheimer's disease (AD) and related dementias (ADRD), pose a growing burden on our rapidly aging society [1, 2]. In 2016, dementia was the fifth leading cause of death around the world [3]. The most common cause of dementia is AD, which is characterized by the presence of extracellular amyloid-β_{1-42} ($A\beta_{1-42}$) plaques and intraneuronal tau aggregations (neurofibrillary tangles, NFTs), among other alterations, that disrupt cell-to-cell communication and transport and trigger pathologic inflammatory processes [4, 5].

Increasing evidence has linked air pollution, such as fine particulate matter ($PM_{2.5}$), to neurodegeneration and ADRD [6–10]. Additionally, several studies have specifically documented aggregations of $A\beta_{1-42}$, standardized AD stages, and other pathologic changes, in brain tissue from children, young adults, and canines exposed to high levels of air pollution [11–14]. Experimental laboratory studies also provide evidence of alterations in levels of AD and related molecular markers after exposure to air pollution and/or inhaled metals [15–17]. Current hypotheses suggest that these central nervous system (CNS) effects of $PM_{2.5}$ and other air pollutants may be mediated through direct and/or indirect pathways leading to oxidative stress and inflammation [18–20].

Despite the growing links between air pollution and neurodegeneration as well as the plausible mechanisms to support these associations, no studies to date have evaluated the association between exposure to $PM_{2.5}$ and AD neuropathology at death in older adults: the primary population affected by AD and one that might be particularly sensitive to the effects of $PM_{2.5}$ due to decreased antioxidant defenses in the aging brain [21, 22]. Most of the human studies conducted to date have focused primarily on children and young adults and/or have been descriptive analyses [11–13, 23]. There is one recent cross-sectional study of adults with mild cognitive impairment or dementia that identified an association between elevated $PM_{2.5}$ and $A\beta$ accumulation assessed via positron emission tomography (PET) scan [24].

To address this gap, we conducted a novel analysis designed to investigate the association between long-term $PM_{2.5}$ exposure and markers of AD neuropathology at autopsy among participants in the Adult Changes in Thought (ACT) prospective community-based cohort study in Seattle, Washington, USA [25]. The results of this pathophysiological-based epidemiological investigation complement prior studies that have evaluated clinical outcomes in AD and expand upon our understanding of the neurotoxic effects of $PM_{2.5}$.

METHODS

Study design

This study was approved by the University of Washington Institutional Review Board. As previously described, the ACT study is a prospective community-based cohort study in Seattle, WA, USA. This cohort is comprised of an urban and suburban elderly (>65 years) population randomly sampled from a well-established health maintenance organization (HMO) (Group Health, now Kaiser Permanente of Washington) [25]. Enrollment of cognitively intact (defined as CASI (Cognitive Abilities Screening Instrument) score of >85 or consensus diagnosis of "not demented" after comprehensive assessment) individuals began in 1994–96 (original cohort, $n = 2{,}581$) and has been expanded to maintain 2,000 person-years at-risk per calendar year. Consent for autopsy is optional and is discussed at study enrollment and follow-up visits. When an individual does consent, it is confirmed by next-of-kin at the time of participant death, as required by Washington State law. As of the most recent ACT study data freeze in September 2018, 5,546 participants have been enrolled, and 832 autopsies have been conducted.

Exposure assessment

We assigned $PM_{2.5}$ exposure uniquely to each participant based on residential addresses geocoded with ArcMap version 10.5 (Redlands, California) for individuals living within the Puget Sound modeling region covered by our spatiotemporal exposure prediction model. We obtained high quality participant address history from billing records starting in 1989;

prior to that date, address data were available from various sources including Group Health/Kaiser Permanente administrative records. Updated addresses, due to participant change of residence, for example, were incorporated when possible. If participants moved out of the spatiotemporal modeling region during the course of our study, no exposure estimates were able to be generated following the move. Additional information on how gaps in address coverage were addressed are detailed in the Supplementary Material.

We estimated annual average $PM_{2.5}$ concentrations based on two-week average concentrations from a hierarchical spatiotemporal prediction model using land use regression (LUR) and geostatistical smoothing, similar to prior published work [26–28]. This new model was developed from $PM_{2.5}$ monitoring data covering the years 1978–2019 across the Puget Sound region in Washington State, including 46 long-term (>2 years) regulatory monitors at 29 sites, 52 sites from research campaigns conducted in 1999–2001 and 2012, and 110 community and study participant home sites (2017–2019) using low-cost sensor measurements (with 5 sites co-located with regulatory monitors). See the Supplementary Material for additional model details. For long-term averages at regulatory monitoring locations, the final model had a cross-validated R^2 (R^2_{CV}) of 0.87 and a root mean square error (RMSE) of 1.29 $\mu g/m^3$; at low-cost measurement sites, the corresponding values were $R^2_{CV} = 0.78$, RMSE = 0.89 $\mu g/m^3$. Using this final model, we had the ability of predict long-term average $PM_{2.5}$ at participant homes from 1978–2018; based on these data, we created different exposure averaging periods of interest for our analyses.

Outcome assessment

Preservation and evaluation procedures for autopsied brain tissues have been described previously [29]. Briefly, neuritic plaque density in the cerebral cortex was assessed using the Consortium to Establish a Registry for AD (CERAD) score (none; sparse; moderate; frequent) [30]. NFT distribution was assessed by Braak staging (I-II; III-IV; V-VI) [31]. Combined AD pathology (plaques and NFTs) was assessed with an ABC score, in line with recent National Institute on Aging-Alzheimer's Association (NIA-AA) recommendations [32, 33]. For tissue collected prior to these 2012 recommendations, the ABC score was simulated based on CERAD and Braak score. Because 'intermediate' or 'high' AD neuropathologic change is considered sufficient explanation for dementia [33], we converted ABC scores to a binary variable ("not/low" versus "intermediate/high") for our inferential analyses. The simulated score was used for all cases as the primary analysis for this endpoint to ensure consistency among participants given the substantial missingness in the raw scores.

Statistical analysis

Accounting for selection bias

Selection bias is a challenge to the generalizability of the analysis [34, 35]. To be included in the autopsy dataset, participants had to pass through several stages of selection: enrollment, consent to autopsy, continuation in the study over the course of life, death, and next-of-kin consent to autopsy. To address this issue, we employed inverse probability weighting (IPW) [36, 37] to create stabilized weights for use in the inferential analyses. IPW creates a pseudo-population that allows us to model what would have happened if all individuals were included in the autopsy cohort.

Because our inferential analysis did not evaluate time-varying covariates and all aspects of selection are known at autopsy, we considered all stages of selection together in one model. Missing values of key covariates were replaced with the mode or mean for selection modeling. (Missingness was less than 7% for each of the covariates). We fit a logistic regression model to estimate the probability of inclusion in the autopsy subset, given a final set of covariates as determined by forward selection based on the following starting covariates obtained at baseline: ACT study cohort, age at baseline, birth cohort, sex, race, educational degree, neighborhood median household income, smoking status, alcohol use, regular exercise, *APOE* $\varepsilon 4$ status, body mass index (BMI), diabetes, cardiovascular disease, hypertension, multivitamin use, self-rated health, and challenges with instrumental activities of daily living (IADL). While nonlinearity was explored for selected variables, such as age, we determined there was no added benefit of these alternatives. Based on the selected model, we computed stabilized weights [36, 38] using sex as the numerator. Stabilization is recommended for IPW because standard, non-stabilized weights could be very large (unstable) for observations with low probabilities [36]. Extreme weights were truncated at 10. The stabilized weights were included as the weight parameter in the inferential analyses.

Regression analyses

We conducted ordinal logistic regression to evaluate the association between long-term exposure to $PM_{2.5}$ and ordered Braak and CERAD stage. We conducted logistic regression to evaluate the association between long-term exposure to $PM_{2.5}$ and dichotomized ABC score (none/low versus intermediate/high). Importantly, we evaluated these outcomes in all individuals, regardless of clinical dementia diagnosis status. Because of the extended period of disease development in AD [39, 40], it is highly relevant to examine these neuropathologies in cognitively normal individuals. Furthermore, based on the cognitive reserve and brain reserve hypotheses, some individuals may appear cognitively normal despite significant AD-related neuropathology [41–45].

We addressed potential confounding with detailed covariate data based on baseline (enrollment) information unless otherwise noted below. The following key covariates and precision variables, with missingness filled in with mean or mode as described for the IPW modeling above, were selected in a tiered model approach: model 1 (M1) (crude/minimally adjusted model): sex [46–48], *APOE* genotype (defined as $\geq$1 ε4 allele versus 0 ε4 alleles) [49–52], age at death [53, 54]; model 2 (M2) (*a priori* main model): M1 + year of death, educational degree [55, 56], neighborhood median household income [57]; model 3 (M3) (extended model): M2 + race [58–60], smoking pack years [61, 62], regular exercise [63, 64]; model 4 (M4) (extended + mediation model): M3 + BMI [65–67], diabetes [68–71], hypertension [72–74], and cardiovascular disease [75–77]. Strong temporal trends in our exposure data informed our decision to use calendar year of death to adjust for confounding by time. B-splines with three degrees of freedom were used to model age at death, year of death, and smoking pack years.

Our primary analyses focused on the association between 10-year average $PM_{2.5}$ and the outcomes of interest, using the M2 covariates described above. We selected this exposure window because it was the longest averaging period for which we had high confidence in our exposure modeling and address history coverage for the cohort; this extended period mirrors the long disease development and progression in ADRD [39, 54]. In secondary analyses, we evaluated alternative exposure averaging periods (1-y; 5-y; 20-y) as well as an exposure period incorporating a lag time (5-y with 10-y lag) given the extended timeline involved in the development of dementia pathologies.

In additional secondary analyses, we evaluated the potential for effect modification between *APOE* genotype status and $PM_{2.5}$ exposure in the *a priori* model. If $PM_{2.5}$ contributes to the development of Aβ plaques and NFTs through oxidative stress and neuroinflammation, then individuals with one or two copies of the ε4 allele may be more susceptible to the neurotoxic effects of $PM_{2.5}$. We also evaluated the sensitivity of our results to the following cohort restrictions: never smokers; non-smoker at baseline; complete address history; and death after year 2000. We also conducted a sensitivity analysis using dichotomous rather than ordered categorical outcomes for Braak and CERAD.

The processes described above for both the selection and regression modeling were implemented on 1,000 bootstrap samples drawn with replacement from the original 5,546 person dataset. Point estimates were calculated by averaging the results of these replicate regression analyses, and confidence intervals were obtained from the 2.5th and 97.5th percentiles of the empirical bootstrap distribution.

All data analysis was performed using R version 4.0.0.

RESULTS

Descriptive statistics

Overall, population characteristics of the autopsy cohort were fairly similar to those of the full ACT cohort (Table 1). However, the autopsy cohort had fewer participants who were in the latest birth cohort (autopsy: 12%; full ACT: 38%); a higher proportion of participants who were white (autopsy: 94%; full ACT: 89%), a higher proportion with $\geq$1 *APOE* ε4 allele (autopsy: 27%; full ACT: 21%); a lower proportion who were obese (autopsy: 26%; full ACT: 31%), and a larger proportion of individuals with a dementia diagnosis (autopsy: 45%; full ACT: 23 %). The average (standard deviation (SD)) age at entry and age at death for individuals in the autopsy cohort was 77 (7) and 89 (7) years, respectively.

Exposure coverage and quality information is provided in the Supplementary Material. Mean (SD) 10-year average $PM_{2.5}$ from death across the autopsy cohort was 8.2 (1.9) $\mu g/m^3$. However, this overall summary masks an important temporal trend across time, as depicted in Fig. 1, with 10-year average $PM_{2.5}$ decreasing over time as expected based on secular trends in air pollution. This strong temporal trend informed our decision to use calendar year of death to

Table 1
Population characteristics of the full cohort and autopsy cohort. Continuous variables presented as mean (standard deviation (SD)); categorical variables presented as n (%)

Mean (SD)/n (%)	Total ($n = 5,546$)	Autopsy ($n = 832$)
Baseline Age (y)	74 (6)	77(7)
Age at Death (y)	87 (7)	89 (7)
ACT Cohort		
Original	2,581 (47%)	512 (61%)
Expansion	811 (15%)	188 (23%)
Replacement	2,154 (39%)	132 (16%)
Birth Cohort		
c.1890–1910	637 (11%)	151 (18%)
1915	783 (14%)	206 (25%)
1920	1,049 (19%)	220 (26%)
1925	973 (18%)	158 (19%)
c.1930–1950	2,104 (38%)	97 (12%)
≥1 *APOE* ε4 allele	1,179 (21%)	224 (27%)
Female	3,228 (58%)	480 (58%)
White	4,956 (89%)	786 (94%)
Census Tract Median Household Income		
<35,000	528 (10%)	77 (9%)
35,000–49,999	1,709 (31%)	262 (31%)
50,000–74,999	2,703 (49%)	414 (50%)
>75,000	606 (11%)	79 (9%)
Degree		
None	465 (8%)	65 (8%)
GED/High School	2,089 (38%)	356 (43%)
Bachelors	1,285 (23%)	197 (24%)
Masters	859 (15%)	109 (13%)
Doctorate	327 (6%)	43 (5%)
Other	521 (9%)	62 (7%)
Smoking Status		
Never	2,706 (49%)	372 (45%)
Past	2,569 (46%)	411 (49%)
Current	271 (5%)	49 (6%)
Regular Exercise	3,969 (72%)	584 (70%)
Body Mass Index (BMI)		
Underweight/Normal	2,014 (36%)	327 (39%)
Overweight	1,836 (33%)	285 (34%)
Obese	1,696 (31%)	220 (26%)
Diabetes	577 (10%)	86 (10%)
Cardiovascular Disease	492 (9%)	92 (11%)
Hypertension	2,284 (41%)	314 (38%)
Avg PM2.5 ($\mu g/m^3$) for the 10-y Prior to Death[1]	9.0(2.4)	8.2(1.9)
Braak stage		
0	-	24 (3%)
B1	-	204 (25%)
B2	-	291 (35%)
B3	-	304 (37%)
Missing	-	9 (1%)
CERAD score		
None	-	187 (22%)
Sparse	-	205 (25%)
Moderate	-	199 (24%)
Frequent	-	238 (29%)
Missing	-	3 (0.4%)
ABC score (simulated)		
None/Low	-	414 (50%)
Interm/High	-	412 (50%)
Missing	-	6 (0.7%)
Dementia diagnosis	1270 (23%)	378 (45%)

[1]Calculated for those 2747 in the cohort who have already died and also have air pollution predictions (out of 2771 deaths)

adjust for confounding by time. Between-year variation (SD: 2.6) was much higher than within-year variation (SD: 0.4) in our dataset.

With respect to the distribution of outcomes in the autopsy cohort, the most common findings were stage B3 Braak stage (37%) and frequent CERAD score (29%). There were equal proportions of individuals in the none/low and intermediate/high (simulated) ABC score groups (50%).

There were an average of 830 autopsies across 1,000 bootstrapped samples of the full dataset. The only variable included in all selection models across these bootstrapped datasets was birth cohort. Other commonly selected variables included: ACT cohort, educational degree, race, *APOE* genotype, smoking status, self-rated health, challenges with IADL, and hypertension. After applying these selection models to the IP weight modeling process and prior to truncation, there were, on average, 6 individuals in each dataset with weights above 10. The mean of stabilized, truncated IP weights across all bootstrapped samples was 0.99, with a range of 0.25 to 10.

Regression analyses

In our *a priori* primary analyses using IP-weighting, we estimated that each 1 $\mu g/m^3$ increase in 10-year average $PM_{2.5}$ prior to death was associated with a suggestive increase in the odds of worse brain pathology as indicated by higher CERAD score (OR: 1.35 (0.90, 1.90)) (Fig. 2). However, there was no association with Braak score (OR: 0.99 (0.64, 1.47)), and $PM_{2.5}$ was suggestively associated with less pathology as indicated by a lower odds of the simulated ABC score (OR: 0.79 (0.49, 1.19)). However, for all outcomes, the confidence intervals were consistent with a range of effects, including no association, and therefore we cannot draw strong conclusions.

Overall, our results were robust to different modeling strategies. Results from crude models were attenuated while results from more richly adjusted models were similar to the primary models. Results from sensitivity analyses were similar to the primary analysis, though the estimate was larger and confidence intervals much wider when the population was restricted to never smokers for CERAD in particular (Supplementary Figure 5). Similarly, while there was some variation in the effect estimates across different exposure averaging periods, the ranges of the confidence intervals were overlapping with the primary analyses (Supplementary Figures 6–8). Overall, IP-weighting showed associations that overlapped

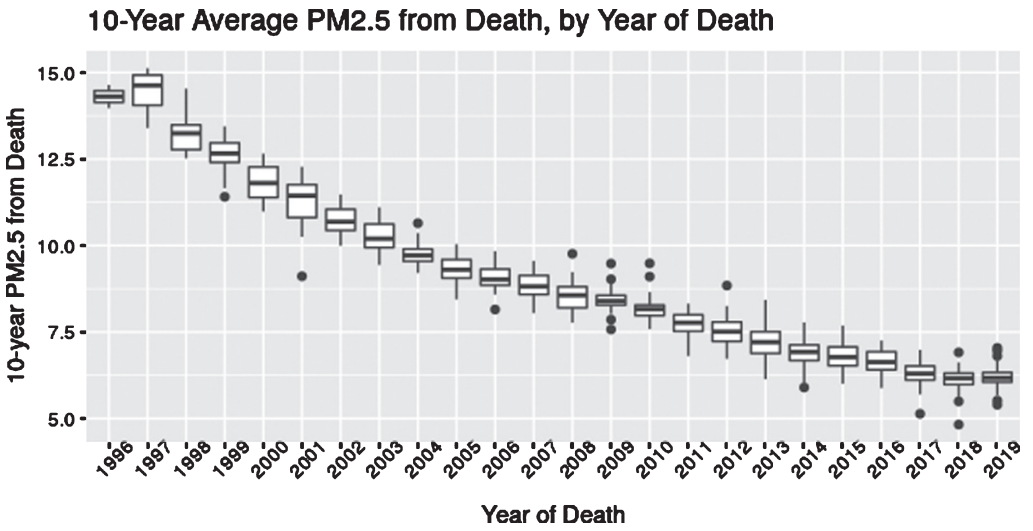

Fig. 1. 10-year average $PM_{2.5}$ exposure by Year of Death in the Autopsy Cohort. In each boxplot, the middle line represents the median value; the edges of the box represent the 25th and 75th percentiles, and the whiskers extended up to 1.5 times the interquartile range (IQR). Points represent outlier observations outside this range.

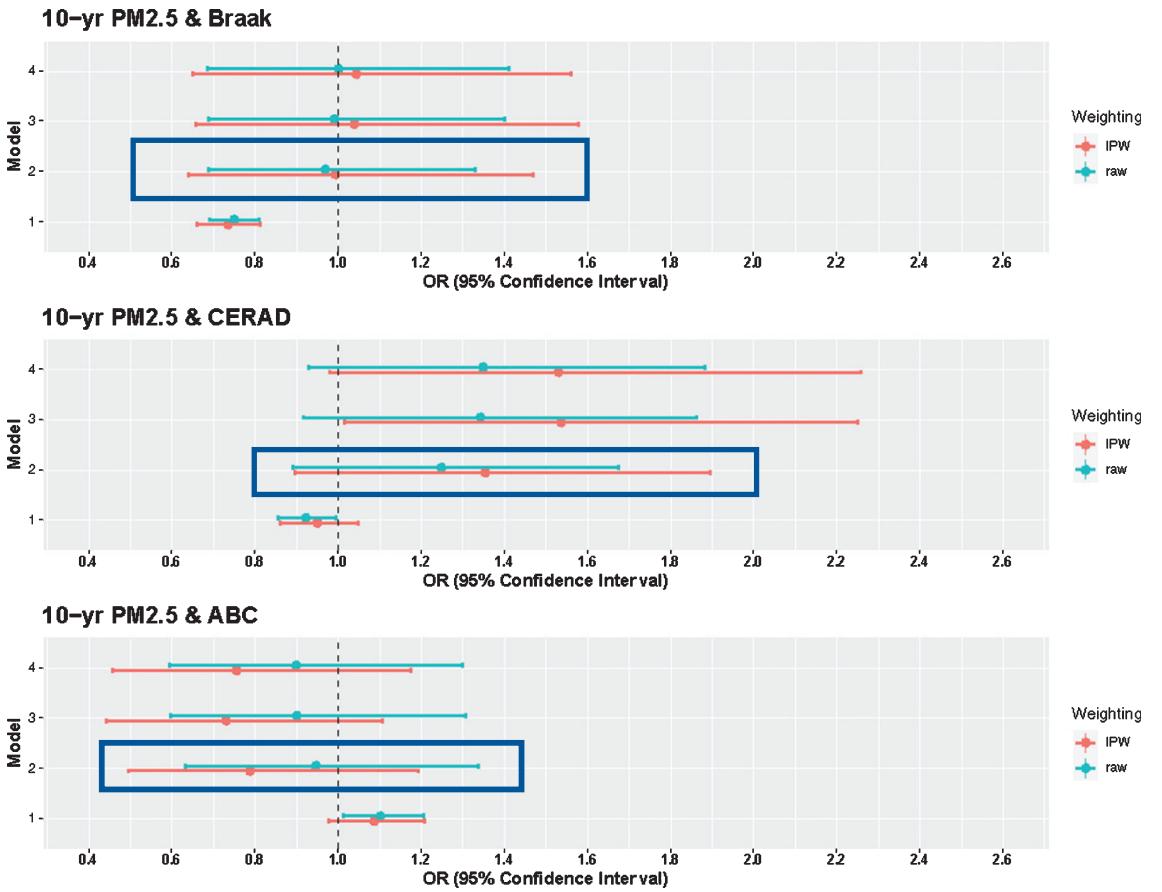

Fig. 2. Association between 1 μg/m^3 increase in 10-year average exposure to $PM_{2.5}$ and AD neuropathology at autopsy. Box indicates *a priori* model. M1: sex, *APOE* ε4 status, age at death; M2 (*a priori*): M1 + year of death, educational degree, neighborhood median household income; M3: M2 + race, smoking pack years, regular exercise; M4: M3 + BMI, diabetes, hypertension, and cardiovascular disease.

considerably with unweighted associations though the IP-weighted estimates had larger confidence intervals. There was no evidence of effect modification by *APOE* genotype (*APOE* interaction *p*-values: Braak = 0.97; CERAD = 0.09; ABC = 0.24).

DISCUSSION

Our study is the first to evaluate the association between $PM_{2.5}$ and AD neuropathologies using autopsy samples in a community-based prospective cohort study comprised of older adults. While specific point estimates for the outcomes of interest suggest potential associations, the results were inconsistent across outcomes and none of the observed associations could be distinguished from no association. These findings were obtained from a population composed predominantly of individuals who self-identified as white, with an average age of nearly 90, and where 94% of the cohort had a 10-year average $PM_{2.5}$ exposure below the current US annual standard of 12 $\mu g/m^3$.

Prior *in vivo* and *in vitro* experimental studies suggest adverse effects of $PM_{2.5}$ on AD neuropathologies. For example, 9-month $PM_{2.5}$ exposure has been shown to cause early AD-related changes and increased expression of pro-inflammatory enzymes in mice [16]. Three-week and six-month exposure to diesel exhaust, a complex mixture of gases and particulates (including $PM_{2.5}$), has been shown to stimulate and/or accelerate AD markers, including plaque formation, in both mice and rats [78, 79]. *In vitro* studies have demonstrated that $PM_{2.5}$ leads to increased Aβ levels in *ex vivo* mouse hippocampal tissue [80] and that nano-size traffic-related PM leads to increases in both oxidative stress and Aβ production in mouse neuronal cells [81].

While there are currently no other prospective community-based cohort studies of air pollution and AD neuropathology in older adults at autopsy to our knowledge, previously published analyses that have documented aggregations of $A\beta_{1-42}$, standardized AD stages, and other molecular changes such as those reflecting oxidative stress, in brain tissue from children, young and middle age adults, and canines exposed to high levels of air pollution [11–14, 23]. Yet, it is important to note that these prior data are based on populations living in Mexico City, which has a yearly average $PM_{2.5}$ concentration of 25 $\mu g/m^3$ [14]. By contrast, our study population experienced lower exposure concentrations: the overall mean (SD) across all years in our autopsy cohort was 8.2 (1.9) $\mu g/m^3$, with a range of 4.8–15.1 $\mu g/m^3$. The overall study mean is below the current U.S. Environmental Protection Agency (EPA) national ambient air quality standard for $PM_{2.5}$ of 12 $\mu g/m^3$, and 94% of the autopsy cohort individuals experienced a 10-year average $PM_{2.5}$ below this level. This may partially explain our inconclusive results: between the lower exposure levels and the relatively small variation of exposure within year (SD: 0.4), we may not have had enough power to estimate effects of the low exposures experienced by our cohort. However, our findings of a suggestive association between $PM_{2.5}$ and CERAD score results are aligned with a recently published cross-sectional study of adults with mild cognitive impairment and dementia. This study of over 18,000 adults experiencing exposures in a similar range to our study population identified associations between increased $PM_{2.5}$ and Aβ accumulation assessed via PET scan [24].

Investigators have used the ACT cohort to evaluate the effect of other exposures on these standardized categorical scores and stages for AD neuropathology, providing another point of comparison for this research. For example, heavy anticholinergic use—which is linked to increased risk of dementia [82]—was associated with a suggestive increase in CERAD score (1.22 (0.81, 1.88)) and a suggestive decrease in Braak stage (0.89 (0.47, 1.66)), but both of these confidence intervals were overlapping with the null. High glucose exposure in the five years prior to death was not associated with elevated Braak stage (RR: 1.06 (0.53, 2.04)) or CERAD score (RR: 1.01 (0.67, 1.51)) [83]. Our effect estimates are in a similar range to these findings from studies also investigating the impact of known dementia risk factors on AD neuropathology. These results could suggest that the elevated dementia risk from $PM_{2.5}$ (or the other risk factors investigated in prior ACT studies) are mediated through mechanisms other than formation of tau tangles and Aβ plaques.

Another relevant comparison may be found in autopsy studies of smoking and AD neuropathology. In an analysis using a smaller sample from the ACT cohort (N = 238), Tsuang et al. reported that compared to moderate smokers (5–50 pack years), never/low smokers (0–5 pack years) had a suggestive elevated risk of higher ABC score (IP-weighted RR: 1.16 (0.55, 2.81)), while heavy smokers (>50 pack years) had a suggestive decreased risk (IP-weighted RR: 0.65 (0.17, 2.25)), though both estimates had large confidence intervals consistent with a range of

effects [84]. In an analysis using the Honolulu-Asia Aging Study with light smokers (0–26.7 pack-years) as the reference category, medium (26.7–40.5 pack-years) and heavy (40.5–55.5 pack-years) smokers were more likely to have higher counts of neocortical neuritic plaques (count ratios (95% CI); medium: 2.12 (1.17, 3.86), heavy: 2.09 (1.14, 3.84)), though this association was attenuated in very heavy smokers (>55.5–156 pack-years) (1.25 (0.60, 2.58)). Associations between smoking intensity and hippocampal NFTs, neocortical NFTs, and hippocampal neuritic plaques, respectively, were mixed, with some effect estimates suggesting adverse effects while other suggesting protective effects; overall, confidence intervals for most of these estimates were overlapping with the null [85]. Most other published studies report null or protective effects of smoking and AD neuropathology [61, 86–88].

A likely reason for the inconclusive and potentially counterintuitive results across all of these smoking studies as well as our study is that these exposures are associated with premature mortality [89], yet age is associated with neuropathology [90]. Therefore, we cannot rule out the possibility that our results are biased due to the fact that age at death is behaving like a quasi-mediator in the association between $PM_{2.5}$ and AD neuropathology. This can also be perceived as a form of selection bias, where individuals who die earlier from higher intensity exposures would exhibit less severe neuropathology because they died younger than other individuals. While most studies of smoking and AD neuropathology, as well as this current analysis of $PM_{2.5}$, have included age at death as a covariate, simple adjustment for this mediator may still lead to bias if there are unmeasured confounders that impact both age at death and the outcome of interest. Advancements in biostatistical methods, including by incorporating tools from the causal inference literature, are needed to address this potential bias and identify alternative estimands that better address the scientific questions at hand.

We did not observe evidence of effect modification by *APOE* genotype. Some prior studies of air pollution and cognitive decline or dementia risk suggests that effects of air pollution exposure may be more pronounced people with ≥ 1 $\varepsilon 4$ allele [11, 12, 91–95], though other studies do not support effect modification by *APOE* genotype [96]. Future studies in the area of air pollution and ADRD should continue to evaluate the potential for interaction by *APOE* genotype.

The central, novel contribution of this work is the evaluation of the association between $PM_{2.5}$ and AD neuropathological stages in a community-based cohort rather than clinical AD diagnosis alone, as has been the focus of prior cohort studies [6–8, 10]. AD neuropathology and AD dementia diagnosis may not be aligned in many individuals [97]. Due to the extended period of disease development in AD, plaques and tangles manifest prior to detectable symptoms [39, 40, 98, 99]. In fact, studies suggest that approximately 20–40% of cognitively normal elderly individuals have significant amyloid plaques [100–104], though this statistic varies by age and *APOE* genotype [103]. Furthermore, based on the cognitive reserve and brain reserve hypotheses, some individuals may appear cognitively normal (i.e., receive no clinical AD diagnosis) despite significant AD-related neuropathology [41–45]. Therefore, a study that directly evaluates AD neuropathology provides different information than one relying on incidence data alone.

To evaluate neuropathology, we used standardized, well-accepted stage and score classifications for AD, which allows for comparison to other published studies. Yet, using continuous measures could provide more precision to answer this scientific question. Future analyses could use quantitative measures of neuropathology, such as histelide [105] or Luminex-based [106] approaches.

A major strength of this work is that we utilized a newly developed spatiotemporal exposure prediction model, specifically for the Puget Sound, that provided estimates of residence-based $PM_{2.5}$ for 40 years (1978–2018). This provides extensive exposure history with which to examine our research question. We complemented this exposure assessment data with detailed address histories available through Group Health/Kaiser Permanente of Washington records, with nearly complete histories since 1989 for the entire cohort and reasonably good coverage prior to 1989. Overall, we were able to estimate 10-year average $PM_{2.5}$ exposures using known address history for 98% of the individuals in the autopsy dataset across the entire study period. Evaluating a long exposure period is crucial for this research question, given the extended period of disease development in ADRD [39]. We acknowledge that our primary time window of 10 years, while an unprecedently long exposure period for this type of analysis, may not capture the etiologically relevant exposure window for AD. In fact, the specific window of vulnerability for AD is still unknown. Our sensitivity analysis investigating a 20-year period provides information on even more extended exposures and suggests similar results to our

primary analysis. Future studies leveraging new technologies may be able to capture lifetime exposures to air pollution to better understand the effects of exposures across the life course on the development of AD neuropathologies.

Yet, there were also challenges in utilizing an extended exposure history. For example, there were limited monitoring sites across the region during the early years; therefore, the spatial contrasts in our model rely heavily on information from more recent years. This issue is especially important to consider given that by adjusting for year of death in our *a priori* and extended models, we are essentially eliminating the larger between-year temporal contrasts and relying entirely on the smaller within-year spatial contrasts for the inferential analyses: the spatial contrasts during earlier periods may have more bias. Additionally, measurement error concerns aside, these low within-year spatial exposure contrasts likely explain the fairly wide confidence intervals for our results.

Another challenge for our study—as in all cohort studies of elderly populations—is selection bias, which occurs with differential enrollment or attrition of study participants. Overall, the ACT study has an exceptional Completeness of Follow-up Index (95.6%) [107], which minimizes our concern with bias due to selective attrition in general. However, differential enrollment into the autopsy cohort, specifically, could still be an issue. An important strength of this work is that we utilized IP-weighting in our inferential models to minimize the impact of this selection bias. In the end, we observed minimal impact of this weighting across our analyses.

We acknowledge that our cohort was comprised of mostly self-identified white, middle class individuals with relatively low rates of co-morbidities; therefore, our results may have limited generalizability. Lack of diversity is a well-recognized problem in ADRD research [108]. Future studies should aim to enroll and follow individuals from more diverse populations. Nevertheless, the ACT study is one of the few true community-based cohort studies of older adults, and the generalizability of results has been excellent to date [25, 109].

In summary, we report suggestive but inconclusive results regarding the association between long-term $PM_{2.5}$ and AD neuropathology. Our results are similar to other studies of known AD risk factors on AD neuropathology [83, 110]. Given the potential bias resulting from mediation by age at death, a future analysis that more appropriately accounts for this factor may provide more accurate effect estimates. Further work is needed in this area, including development of appropriate statistical methods, given the growing evidence of the association between long-term exposure to $PM_{2.5}$ and clinical AD [7].

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SUPPLEMENTARY MATERIAL

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