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Outdoor air pollution and histologic composition of normal breast tissue

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Abstract

Background: Biologic pathways underlying the association between outdoor air pollution and breast cancer risk are poorly understood. Breast tissue composition may reflect cumulative exposure to breast cancer risk factors and has been associated with breast cancer risk among patients with benign breast disease. Herein, we evaluated whether fine particulate matter ($PM_{2.5}$) was associated with the histologic composition of normal breast tissue.

Methods: Machine-learning algorithms were applied to digitized hematoxylin and eosin-stained biopsies of normal breast tissue to quantify the epithelium, stroma, adipose and total tissue area from 3,977 individuals aged 18–75 years from a primarily Midwestern United States population who donated breast tissue samples to the Susan G. Komen Tissue Bank (2009-2019). Annual levels of $PM_{2.5}$ were assigned to each woman's residential address based on year of tissue donation. We applied predictive *k*-means to assign participants to clusters with similar $PM_{2.5}$ chemical composition and used linear regression to examine the cross-sectional associations between a 5-μg/m^3 increase in $PM_{2.5}$ and square root-transformed proportions of epithelium, stroma, adipose, and epithelium-to-stroma proportion [ESP], overall and by $PM_{2.5}$ cluster.

Results: Higher residential $PM_{2.5}$ was associated with lower proportion of breast stromal tissue [β=−0.93,95% confidence interval: (−1.52, −0.33)], but was not related to the proportion

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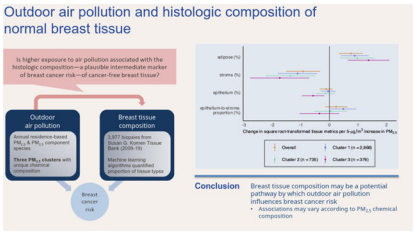
of epithelium [β=−0.11 (0.34, 0.11)]. Although $PM_{2.5}$ was not associated with ESP overall [β=0.24 (−0.16, 0.64)], the association significantly differed by $PM_{2.5}$ chemical composition (*p*-interaction=0.04), with a positive association evident only among an urban, Midwestern cluster with higher concentrations of nitrate (NO_3^-) and ammonium (NH_4^+) [β=0.49 (0.03, 0.95)].

Conclusions: Our findings are consistent with a possible role of $PM_{2.5}$ in breast cancer etiology and suggest that changes in breast tissue composition may be a potential pathway by which outdoor air pollution impacts breast cancer risk. This study further underscores the importance of considering heterogeneity in $PM_{2.5}$ composition and its impact on breast carcinogenesis.

Graphical Abstract



Keywords

Air pollution; Particulate matter; Breast cancer; K-means clustering

1. Background

Existing evidence suggests that exposure to ambient air pollution is associated with an increased risk of breast cancer. For example, nitrogen dioxide (NO_2) and polycyclic aromatic hydrocarbons (PAHs), markers of traffic-related air pollution, have consistently been associated with elevated breast cancer risk (Cheng et al. 2020; Datzmann et al. 2018; Gabet et al. 2021; Niehoff et al. 2019; White et al. 2018). Studies of outdoor exposure to particulate matter less than 2.5 microns in diameter ($PM_{2.5}$) and breast cancer published prior to 2018 have reported largely null results (Andersen et al. 2017a; Andersen et al. 2017b; Hart et al. 2016; Reding et al. 2015). However, subsequent studies that have considered geographic heterogeneity in the composition of $PM_{2.5}$ have observed evidence of a positive association (Villeneuve et al. 2018; White et al. 2021; White et al. 2019a). For example, higher exposure to $PM_{2.5}$ in two large, United States (US)-based prospective cohort studies was associated with a higher risk of breast cancer, with findings varying according to geographic region, suggesting that varying $PM_{2.5}$ chemical composition is an important consideration when evaluating the role of $PM_{2.5}$ in breast cancer (White et al. 2021; White et al. 2019a).

Studies of air pollution and breast density further support a role of air pollution in breast cancer etiology (DuPre et al. 2017; Huynh et al. 2015; Yaghjyan et al. 2017). Increased mammographic breast density—a measure of the relative proportion of fibroglandular to adipose tissue in the breast—is a well-established breast cancer risk factor (Nazari and Mukherjee 2018). Among individuals who underwent mammography screening within the US Breast Cancer Surveillance Consortium, those with dense breasts were more likely to

have higher residential exposure to $PM_{2.5}$ (Yaghjyan et al. 2017) and PAHs (White et al. 2019c). Additionally, our group previously found that residential $PM_{2.5}$ may impact normal breast tissue characteristics through its association with reduced involution of terminal duct lobular units (TDLUs) (Niehoff et al. 2020), the epithelial structures where most breast cancers arise (Russo et al. 2000), and the reduction of which in benign breast biopsies has been associated with increased breast cancer risk (Figueroa et al. 2014; Milanese et al. 2006). Together these findings support the continued investigation of breast tissue composition to inform biologic pathways underlying the association between air pollution and breast cancer.

Emerging data suggest that the relative amount of fibroglandular tissue that is epithelium versus stroma may be an important contributing factor to breast cancer development (Abubakar et al. 2021; Vellal et al. 2021). Using novel machine learning-based methods, Abubakar et al. (2021) and Vellal et al. (2021) showed that among independent study populations of individuals with benign breast disease (BBD), the relative proportion or ratio of epithelium-to-stroma was associated with subsequent invasive breast cancer risk, suggesting that the interplay between epithelial and stromal tissue may create conditions that protect against or favor carcinogenesis (Abubakar et al. 2021; Vellal et al. 2021). Abubakar et al. (2023) also demonstrated that these same histologic features within the normal breast were related to established breast cancer risk factors, including obesity and breastfeeding. Hence, quantitative changes in breast tissue composition might constitute plausible intermediate markers of breast cancer risk that can be used to interrogate the potential biologic pathways underlying the associations between environmental exposures and breast cancer. In the present study, we evaluated how residential outdoor $PM_{2.5}$ exposure is related to quantitative tissue composition metrics of the normal breast, with considerations of how associations vary according to $PM_{2.5}$ chemical composition.

2. Methods

2.1 Study Population

A total of 4,722 individuals enrolled in and donated breast tissue to the Susan G. Komen Tissue Bank—a biorepository of breast tissue donated by healthy volunteers—between 2009–2019 (https://komentissuebank.iu.edu/). For participants who donated multiple samples, we considered the earliest instance of tissue donation. We excluded participants without available digitized images of hematoxylin and eosin-stained biopsies (n = 345), with a personal history of breast cancer (n = 163), who were pregnant or breastfeeding at the time of donation (n = 57), who did not have information on menopause status (n = 13) or who were 75 years of age or older (n = 43). We also excluded participants for whom we were unable to assign annual $PM_{2.5}$ estimates (n = 66) either because they did not provide a donation address, or their donation address was not within the contiguous US. We also excluded participants who were missing covariate information used for adjustment in this study (n = 65). Thus, the final sample included in our analysis consisted of 3,977 participants.

At the time of tissue donation, enrollees completed a questionnaire that ascertained information about demographics, medical history, lifestyle factors, and reproductive history.

The protocol was approved by the Indiana University Institutional Review Board. All participants provided written informed consent prior to enrollment.

2.2 Exposure assessment

We estimated annual levels of $PM_{2.5}$ at each participant's residential address corresponding to year of breast tissue donation using the US Environmental Protection Agency's Air Quality Time Series Project (EQUATES), a Bayesian space-time downscaling model that combines modeled air pollution levels from the Community Multiscale Air Quality (CMAQ) Modeling System (version 5.3.2) with fixed site air pollution measurements from the National Air Monitoring Stations (US Environmental Protection Agency 2021). Annual average $PM_{2.5}$ concentrations, created by combining daily averages estimated at the 12 × 12 km grid level, were linked to participant's geocoded residential addresses corresponding to the respective year of tissue donation.

We also considered 2009 annual average concentrations of $PM_{2.5}$ component species sulfate (SO_4^{2-}), nitrate (NO_3^-), ammonium (NH_4^+), elemental carbon (EC), and organic carbon (OC), which were estimated using CMAQ v5.3.2. These five components account for approximately 80% of $PM_{2.5}$ total mass on average in the US, and the relative composition of $PM_{2.5}$ components was stable across the 11year study period (Bell et al. 2007).

2.3 Outcome assessment

The procedure for breast tissue collection involved the removal of up to four tissue cores from the left or right upper outer quadrant of the breast with a standard 9-gauge needle. One core was fixed in 10% buffered formalin or Paxgene, sectioned, and stained with hematoxylin and eosin (H&E). The stained tissue sections were then digitized at 20X magnification. Additional details are described by Figueroa et al. (2014). Next, machine-learning algorithms were applied to quantify histologic tissue composition. Briefly, a 85-datapoint tissue classifier script was trained by two pathologists with expertise in digital pathology and applied to digitized H&E-stained slide images to identify and quantify (in mm^2) the area on each slide comprised of epithelium, stroma, and adipose tissue (Abubakar et al. 2023). The pathologists who performed the algorithm training and image analysis were blinded to all participant characteristics and used Halo Client computational pathology software (Indica Labs, Albuquerque, NM). Previous reproducibility analyses, in which another pathologist independently developed a script to analyze a subset of the images, showed excellent agreement for all three tissue types (Spearman's rho ≥ 0.95). Additional details regarding the algorithm, training, and reproducibility are described in Abubakar et al. (2021); (2023).

Percent epithelium, stroma, and adipose tissue area were calculated by dividing the absolute value of each histologic metric by total tissue area on the slide and multiplying by 100. We calculated an integrated measure of tissue characteristics, percent epithelial-to-stroma proportion (ESP), which was defined by dividing the epithelial area by total fibroglandular tissue area (i.e., epithelium and stroma) and multiplying by 100.

2.4 Statistical analysis

As in Niehoff et al. (2020), we identified groups of individuals with similar $PM_{2.5}$ component profiles using *K*-means clustering, an unsupervised algorithm that assigns observations in a dataset into a pre-specified number of groups (*k*), such that within-cluster variation is minimized and between-cluster variation is maximized (Gibson et al. 2019). To determine the *k*-means clusters, we used the ratio of each component to $PM_{2.5}$ total mass. We identified the optimal number of clusters using two methods: 1) plotting the total within-cluster sum of squares according to number of clusters and determining at which point the plot "bends" (i.e., the "elbow method") and 2) by determining the number of clusters with the highest Rand Index, a measure of the robustness of the clustering algorithm to variability within the population (Hubert and Arabie 1985). Both methods indicated the optimal number of clusters as three.

We evaluated the distribution of participant characteristics, geographic variability, and annual $PM_{2.5}$ exposure overall and by $PM_{2.5}$ component clusters. We used linear regression to model coefficients (β) and 95% confidence intervals (CI) for the association between a 5-$\mu g/m^3$ increase in annual $PM_{2.5}$ total mass concentrations and each tissue composition metric. We applied a square root transformation to tissue composition metrics (i.e., %ESP, %epithelium, %stroma, and %adipose tissue) in order to improve approximate homoskedasticity and normality of model residuals. Thus, the effect estimates can be interpreted as the association between a 5-$\mu g/m^3$ increase in annual $PM_{2.5}$ total mass concentrations and the square root-transformed proportion of epithelium, stroma, adipose, epithelial-to-stromal proportion. We also explored associations between concentrations of each $PM_{2.5}$ component species and each tissue metric.

To identify potential confounders, we created a directed acyclic graph (DAG) based on previous literature (Greenland et al. 1999). Our adjustment set included the following variables: age at donation (years), smoking status (never, former, current), education (high school graduate or less, vocational/technical/associate degree, college degree, graduate/professional degree), self-identified race/ethnicity (Asian, Hispanic, non-Hispanic Black, non-Hispanic white, other [Native American/Alaskan,Native Hawaiian, Pacific Islander, other, or unknown]), body mass index (BMI, kg/m^2), US census region (Midwest, Northeast, South, or West) and donation year. For models of epithelium-to-stroma proportion (ESP), we additionally adjusted for the percent adipose tissue on the slide.

Comparing models with and without cross-product terms and using a likelihood-ratio test, we considered whether associations varied by $PM_{2.5}$ component cluster assignment and menopause status. Individuals were classified as postmenopausal (n = 1,367) if they reported any of the following: did not have a menstrual period in the 12 months prior to tissue donation, had a bilateral oophorectomy, had a hysterectomy without a bilateral oophorectomy and were ≥ 55 years of age, or had a uterine ablation and were ≥ 55 years of age.

In sensitivity analyses, we explored overall and cluster-specific associations with average 2009 $PM_{2.5}$ total mass concentrations assigned to all participants rather annual concentration based on participants' donation year. We also explored whether associations differed

according to BMI categories and parity using the same methods described in the paragraph above and considered additional adjustment for area-level factors (i.e., census tract-level income and urbanicity; n = 1 missing) and for reproductive history (i.e., parity and breastfeeding; n = 169 missing). Lastly, to rule out any potential influence of cigarette smoking in observed associations, we restricted the analysis to participants who reported never smoking cigarettes. All analyses were carried out using R version 4.2.0 R Foundation for Statistical Computing, Vienna, Austria).

3. Results

The demographic characteristics of participants at the time of tissue donation are summarized in Table 1. Participants were, on average, about 44 years of age. Approximately 18% and 68% were non-Hispanic Black and non-Hispanic White, respectively. The majority of participants had at least a college degree (58%), were overweight or obese (69%), had never smoked cigarettes (73%), and were premenopausal (66%). While the residential locations of participants represent all four US census regions of the contiguous US, most resided in the Midwest (84%) which is reflective of the location of most tissue collection events. The distribution of each tissue metric is presented in Supplemental Table 1. Briefly, breast tissue samples were largely comprised of adipose tissue (81%), followed by stroma (17%) and epithelium (1%). ESP had a moderate to low correlation with individual tissue metrics (r = 0.14 to 0.57; Supplemental Table 1).

The average annual residential level of $PM_{2.5}$ was 11.5 μg/m^2 (interquartile range [IQR] = 5.59, 14.5). We identified three clusters of participants with similar profiles of $PM_{2.5}$ chemical composition (Figure 1). The relative contributions of each component species to $PM_{2.5}$ total mass within each cluster are described in Figure 2. Clusters 1 (n = 2,966) and 2 (n = 376) had similar levels of residential exposure to $PM_{2.5}$ total mass (Cluster 1: mean [IQR] = 11.5-μg/m^3 [6.75, 14.5]; Cluster 2: 11.0-μg/m^3 [5.59, 14.1]), while participants in Cluster 3 (n = 735) had the lowest $PM_{2.5}$ exposure levels overall (9.7-μg/m^3 [6.05,12.5]) and a greater amount of variability in individuals' level of exposure to each component species. While clusters 1 and 2 were similar in regard to concentrations of OC, EC, and SO_4^{2-}, cluster 1 had higher relative concentrations of NO_3^- and NH_4^+ (Figure 2).

When considering tissue types individually, among all participants, a 5-μg/m^3 increase in $PM_{2.5}$ was associated with a lower proportion of stroma tissue (β = −0.95, 95% confidence interval (CI): −1.54–0.35) and, given that stroma and adipose proportion were perfectly negatively correlated (Pearson's r = 1.0; Supplemental Table 1), we also observed a positive association between $PM_{2.5}$ and adipose tissue proportion (β = 0.77, 95% CI: 0.35, 1.19). Neither association meaningfully differed by menopause status or cluster group (Table 2).

Overall, we observed a positive but statistically non-significant positive association between a 5-μg/m^3 increase $PM_{2.5}$ and square root-transformed %ESP (β = 0.23, 95% CI: −0.17, 0.63; Table 2). The association did not differ by menopause status (p-interaction = 0.81), but there was significant heterogeneity when considering the association by $PM_{2.5}$ component clusters (p-interaction = 0.04). Higher residential levels of $PM_{2.5}$ were associated with greater %ESP in an urban, Midwestern cluster with higher relative concentrations of NO_3^-

and NH_4^+ (Cluster 1: β = 0.48, 95% CI: 0.02, 0.94). Among participants living in urban areas outside of the Midwest with lower relative concentrations of SO_4^{2-} and NH_4^+, we observed an inverse but imprecise association with %ESP (Cluster 3: β = −0.32, 95% CI: −1.02, 0.37), whereas the association was negligible among a more suburban cluster comprised of individuals living primarily in the Midwest and South (Cluster 2: β = 0.04, 95% CI: −0.54, 0.62; Table 2). Regarding the proportion of epithelial tissue, we observed an inverse association with $PM_{2.5}$ within cluster 3 (β = −0.58, 95% CI: −0.98, −0.19) and null associations within cluster 1 (β = −0.05, 95% CI: −0.31, 0.21) and cluster 2 (β = −0.14, 95% CI: −0.47, 0.19; *p*-interaction = 0.04; Table 2).

The associations between a 1-ug/m^3 increase in $PM_{2.5}$ component species and the square roottransformed proportion each tissue metric are presented in Supplemental Table 2. The magnitude and direction of associations for each tissue metric were not consistent across $PM_{2.5}$ component species. Compared to associations with total $PM_{2.5}$ mass, we observed similar associations for NH_4^+ and OC with % epithelium and SO_4^{2-} and OC with % stroma.

The associations stratified according to BMI categories and parity are presented in Supplemental Table 2. Across categories of BMI (i.e., < 25, 25-< 30, and ≥ 30 kg/m^2), the direction of associations between $PM_{2.5}$ and each tissue metric were consistent overall. There was a significant interaction between $PM_{2.5}$ and BMI in relation to the proportion of epithelial and stromal tissue area (*p*-interaction = 0.04 and 0.05, respectively) but not in relation to the proportion of adipose tissue area or %ESP (*p*-interaction = 0.30 and 0.89, respectively). Among participants with a BMI < 25 kg/m^3 and ≥ 30 kg/m^3, the magnitude of the association between $PM_{2.5}$ and % stroma was more pronounced compared to the overall association (< 25 kg/m^2: β = −1.08, 95% CI: −1.76, −0.39 and ≥ 30 kg/m^2: β = −1.26, 95% CI: −1.99, 0.53), and among overweight participants (BMI > 30), the association was smaller in magnitude and not statistically significant (β = −0.53, 95% CI: −1.21, 0.15). Although there was a significant interaction observed in relation to the proportion of epithelial tissue, none of the associations within strata of BMI were statistically significant. Findings did not substantially differ according to parity, nor did they meaningfully change when adjusting for area-level factors or reproductive history (Supplemental Table 2). Further, we observed similar results when assigning exposure based on 2009 $PM_{2.5}$ concentrations and when restricting the analysis to never smokers (Supplemental Table 2).

4. Discussion

In this cross-sectional study of breast cancer-free individuals who donated breast tissue, we observed that participants with higher residential levels of fine particulate matter were more likely to have breast tissue characterized by a lower proportion of stromal tissue. Further, among those with residential $PM_{2.5}$ with greater relative concentrations of nitrogenous compounds, $PM_{2.5}$ levels were positively associated with epithelium-to-stroma proportion. Elevated ESP has been suggested as an early marker of breast cancer risk among BBD patients. Thus, our findings relating $PM_{2.5}$ to histologic composition of the normal breast may inform early carcinogenic mechanisms by which air pollution influences breast cancer

risk. Our findings also highlight the continued need to consider the variability in $PM_{2.5}$ chemical composition in relation to breast cancer-related outcomes.

$PM_{2.5}$ is a heterogenous mixture comprised of diverse chemical components, including those with both carcinogenic and endocrine disrupting properties. In addition to the five components species considered in our analysis, $PM_{2.5}$ contains compounds with demonstrated estrogenic effects, including metals and PAHs (Choe et al. 2003; Santodonato 1997). While these compounds account for a relatively small proportion of $PM_{2.5}$, they can exert adverse effects even at low levels of exposure. Experimental evidence suggests that tissue organization and the cross talk between epithelial and stromal tissue—important for maintaining constraints on epithelial proliferation (Maffini et al. 2004; Rønnov-Jessen et al. 1996)—is sensitive to exogenous compounds with endocrine disrupting properties (Burks et al. 2017; Macon and Fenton 2013).

Among individuals with benign breast disease, researchers have observed that the proportion or ratio of epithelium-to-stroma was more strongly associated with subsequent invasive breast cancer risk than the proportion of epithelium alone. Vellal et al. (2021) found that a 10-fold increase in the ratio of epithelium to stroma was associated with approximately 30% higher odds of developing breast cancer (OR = 1.29, 95% CI: 1.05, 1.59). Similarly, Abubakar et al. (2021) found that women in the highest quartile of histologic-ESP had two times the odds of developing breast cancer relative to women in the lowest quartile, suggesting that high ESP is a feature of breast tissue composition that is conducive for epithelial proliferation and tumor initiation. Further, an increasing proportion of stromal tissue was associated with reduced breast cancer risk, particularly in the context of non-proliferative BBD (Abubakar et al. 2021), suggesting that the stroma has a protective effect against breast carcinogenesis during normal homeostasis and that stromal depletion may be one mechanism leading to increased ESP. Future work is needed to replicate these findings among individuals without a history of breast disease. While our results suggest that $PM_{2.5}$ exposure is associated with differential histology of the breast, it is not yet clear whether a certain threshold in the proportion of stromal tissue or ESP is most influential for future breast cancer risk. However, we do note that the magnitude of the associations observed here between $PM_{2.5}$ and ESP is comparable to that observed for established breast cancer risk factors such as elevated BMI and breastfeeding history (Abubakar et al. 2023). Thus, our findings of decreased proportion of stromal tissue and greater ESP among individuals with higher residential levels of $PM_{2.5}$ suggests that a plausible pathway by which $PM_{2.5}$ influences breast cancer risk is through contributing to stromal depletion and a concomitant increase in ESP.

Our findings build upon previous work conducted in a subset of participants in the Komen Tissue Bank in which increasing concentrations of residential $PM_{2.5}$ exposure was associated with higher TLDU count. Given that TLDU involution is reflective of a reduction in the amount of epithelial tissue on BBD biopsies (Abubakar et al. 2021), higher TDLU count may reflect a greater amount of epithelium that is susceptible to carcinogenesis. In this previous work, Niehoff et al. (2020) examined tissue samples from a subset of Komen Tissue Bank who enrolled between 2009–2011. Given the labor-intensive nature of TDLU assessment, TDLU involution metrics were not available for all donors in our

sample of participants enrolled up to 2019, and we were thus unable to replicate these previous findings. Nonetheless, our analysis utilizing machine learning-based methods more amenable to larger scale studies provides additional support that fine particulate matter air pollution may influence breast histologic characteristics in this larger population. This was particularly evident among individuals living in urban centers in the Midwest for whom ammonium and nitrate particles contributed to a greater proportion of residential $PM_{2.5}$ exposure. We note, however, that the present analysis and the study by Niehoff et al. (2020) are cross-sectional. Future research is needed to prospectively the examine the association between air pollution exposures and breast tissue composition.

Although our study is the first to examine quantitative histologic breast tissue composition in relation to particulate matter air pollution, our findings can be discussed in the context of previous work investigating $PM_{2.5}$ and mammographic density. Positive associations have been observed with categorical measures of breast density in relation to ambient $PM_{2.5}$ (Yaghjyan et al. 2017) and specific air toxics that are constituents of $PM_{2.5}$ (White et al. 2019c). Among participants 40 years or older in the Breast Cancer Surveillance Consortium (n = 279,967, 2001–2009), Yaghjyan et al. (2017) reported that compared to individuals with scattered fibroglandular breasts, those with heterogeneously dense breast had higher exposure to $PM_{2.5}$ (fourth vs. first quartile odds ratio (OR) = 1.19, 95% CI: 1.16, 1.23). In the same study population, White et al. (2019c) found residential exposure to the airborne heavy metals arsenic, cobalt, lead, manganese, and nickel, both singly and in combination, was associated with greater breast density. Unlike mammographic density, the histologic tissue metrics used in our analysis distinguish between relative amounts of epithelial and stromal tissue. Thus, our findings complement the literature related to breast density and provide additional insight into potential biologic pathways through which air pollution may influence breast tissue composition and subsequent breast cancer risk. Notably, Abubakar et al. (2021) found that ESP and mammographic density were independent and jointly associated with breast cancer incidence. A potential direction for future research would be to examine the association between air pollution and combined measures of breast density and ESP.

In contrast to findings for NO_2, studies for $PM_{2.5}$ and breast cancer risk have been more inconsistent with few considering the role of $PM_{2.5}$ components (Andersen et al. 2017b; White et al. 2019a) or geographic heterogeneity in $PM_{2.5}$ (White et al. 2021; White et al. 2019a). Findings from two large, prospective US-wide cohorts highlight the importance of geographic heterogeneity in $PM_{2.5}$ in relation to breast cancer. In the Black Women's Health Study, increased risk of negative estrogen receptor status (ER-) breast cancer and premenopausal breast cancer was found only among participants in the Midwest (White et al. 2021). In the Sister Study, a cohort of women with a sister with breast cancer who themselves are cancer-free at baseline, White et al. (2019a) observed that higher residential levels of $PM_{2.5}$ were associated with greater incidence of ductal carcinoma in situ (DCIS) breast cancer among participants in the Midwest and a greater incidence of invasive breast cancer among participants in the Western US. Similarly, in the Nurses' Health Study, a prospective cohort of female US nurses established in 1976, DuPre et al. (2017) found no overall association between $PM_{2.5}$ and a continuous measure of mammographic density

although a positive association was suggested among women living in the Northeast (β = 3.9% per 10-μg/m^3 increase in $PM_{2.5}$, 95% CI: −0.02, 7.9).

The relative distribution of $PM_{2.5}$ constituent species are known to vary by geographic regions due to different $PM_{2.5}$ sources (Bell et al. 2007) and thus could contribute to geographic heterogeneity in breast cancer risk. In our study, we identified a Midwest-based cluster (cluster 1) capturing participants living in urban areas among whom we observed a positive association between $PM_{2.5}$ total mass and%ESP. The profile of $PM_{2.5}$ component species may be indicative of $PM_{2.5}$ sources giving rise to the higher proportions of ammonium and nitrate observed within this cluster. For example, in addition to fossil fuel combustion, agricultural activity is a major source of ammonia emissions and the subsequent formation of nitrate and ammonium particles (Kundu and Stone 2014). However, the associations between levels of each individual $PM_{2.5}$ component species and %ESP does not support the hypothesis that is any single component is driving the association with $PM_{2.5}$ total mass. While the five components considered in our analysis together comprise the majority of $PM_{2.5}$ total mass, it is possible that the $PM_{2.5}$ component clusters we identified are proxies of unmeasured components or other air pollutants relevant to effects on the breast. For example, compared to other census regions, the Midwest generally has higher levels of industrial emissions. Monitoring data suggests that $PM_{2.5}$ in the Midwest is comprised of higher levels of heavy metals, including cadmium, vanadium, arsenic, and cobalt (Keller et al. 2017), and higher levels of exposure to these toxic air pollutants have been associated with increased breast density (White et al.2019c) and breast cancer incidence (Andersen et al. 2017b; White et al. 2019b).

Contrary to our hypothesis, residential $PM_{2.5}$ was inversely, but imprecisely, associated with %ESP and the proportion of epithelial tissue among participants assigned to cluster 3. $PM_{2.5}$ exposure within this cluster can be characterized as having lower proportion of sulfate particles which may indicate fewer emissions from fossil fuel combustion including those from coal-burning power plants (Bell et al. 2007). However, one of the most defining features of cluster 3 was that it was comprised of a limited number of individuals living outside of the Midwestern US, including participants in both Southern California and New York. Given that areas on the West and East coast have different air pollution profiles (Bell et al. 2007), cluster 3 is characterized by greater heterogeneity in the levels of each $PM_{2.5}$ component species compared to the other clusters, as shown in Figure 2. Thus, it is unclear whether cluster 3 represents a meaningful profile of $PM_{2.5}$ chemical composition and whether any true associations may be obscured given the heterogeneity within the cluster.

The findings of this study should be interpreted considering the strengths and limitations of our exposure assessment methods. The use of high-quality air pollution data estimated using the ESCAPE model—a technique integrating robust modeling procedures with air pollution measurements—is an important strength of our study. Also, using modelled estimates of $PM_{2.5}$ component species allowed us to consider the impact of heterogeneity in $PM_{2.5}$ chemical composition. Although we assigned 2009 levels of component species to all participants regardless of donation year, the relative composition of $PM_{2.5}$ was stable during the study period. In addition, we estimated annual concentrations of $PM_{2.5}$ based on

participants' home residence, which does not capture indoor air pollution nor time spent away from the home that could impact their exposure. We expect resulting misclassification of exposure, however, to be non-differential with respect to breast tissue composition. Importantly, we did not have information on how long participants had lived at their address reported at the time of tissue donation or information on past residences. Thus, we were not able to evaluate long-term air pollution exposure or exposure during potentially sensitive periods in which changes in breast composition may be vulnerable to air pollution, for example, during fetal development, puberty, or pregnancy (Terry et al. 2019). Current breast tissue composition may more strongly reflect the development and remodeling of the breast during these periods rather than the influence of more recent exposures. Yet recent exposures may still have an important impact; observational studies have demonstrated breast density declines after discontinuation of hormone replacement therapy or tamoxifen use (Chow et al. 2000; Cuzick et al. 2004; Rutter 2001), highlighting the potential modifiability of this breast cancer risk factor even later in life.

Our study leveraged objective and novel quantitative histologic tissue composition metrics among a unique resource of tissue samples from individuals without a personal history of breast cancer. Given that the procedure for collection of breast tissue cores was non-targeted, we cannot rule out the possibility of non-representative sampling of breast tissue and the influence of random error in our effect estimates. However, analyzing tissue component measures as a percentage of the total tissue area helps address this limitation. Additionally, we recognize that applying a square root transformation to the outcome measurements can add difficulty in interpreting the effect estimates. However, regardless of any transformation applied to the tissue metrics, the magnitude of the effect estimates can change depending on the distribution of the tissue metrics in a given population. Thus, we emphasize the direction rather than the magnitude of the observed associations. Given regional differences in obesity (Myers et al. 2015), which is reflected by differences between clusters in the percent adipose tissue in breast tissue samples among our study population, we cannot rule out residual confounding by BMI or adiposity. We sought to minimize this concern by controlling for a continuous measure of BMI and in models examining %ESP, controlling for the proportion of adipose tissue on the slide. We also found limited evidence that associations between $PM_{2.5}$ and tissue composition meaningfully differed by BMI categories. Lastly, the generalizability of our findings may be limited given that participants donated to the Komen Tissue Bank on a volunteer basis and most resided in the Midwestern US, which limited our ability to explore $PM_{2.5}$ component profiles outside of the Midwest.

In conclusion, we observed that higher residential exposure to fine particulate matter with greater relative proportions of nitrogenous compounds was associated with breast histologic characteristics that are indicative of a higher underlying breast cancer risk. Thus, our findings support the possible role of $PM_{2.5}$ in breast cancer etiology and suggest that breast tissue composition may be a potential pathway by which outdoor air pollution impacts breast cancer risk.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- We evaluated air pollution and markers of breast cancer risk in cancer-free tissue
- $PM_{2.5}$ levels were positively associated with epithelium-to-stroma proportion
- $PM_{2.5}$ levels were inversely associated with proportion of stromal tissue area
- Associations with $PM_{2.5}$ varied according to its chemical composition
- Breast histology is a potential pathway by which $PM_{2.5}$ may influence breast cancer

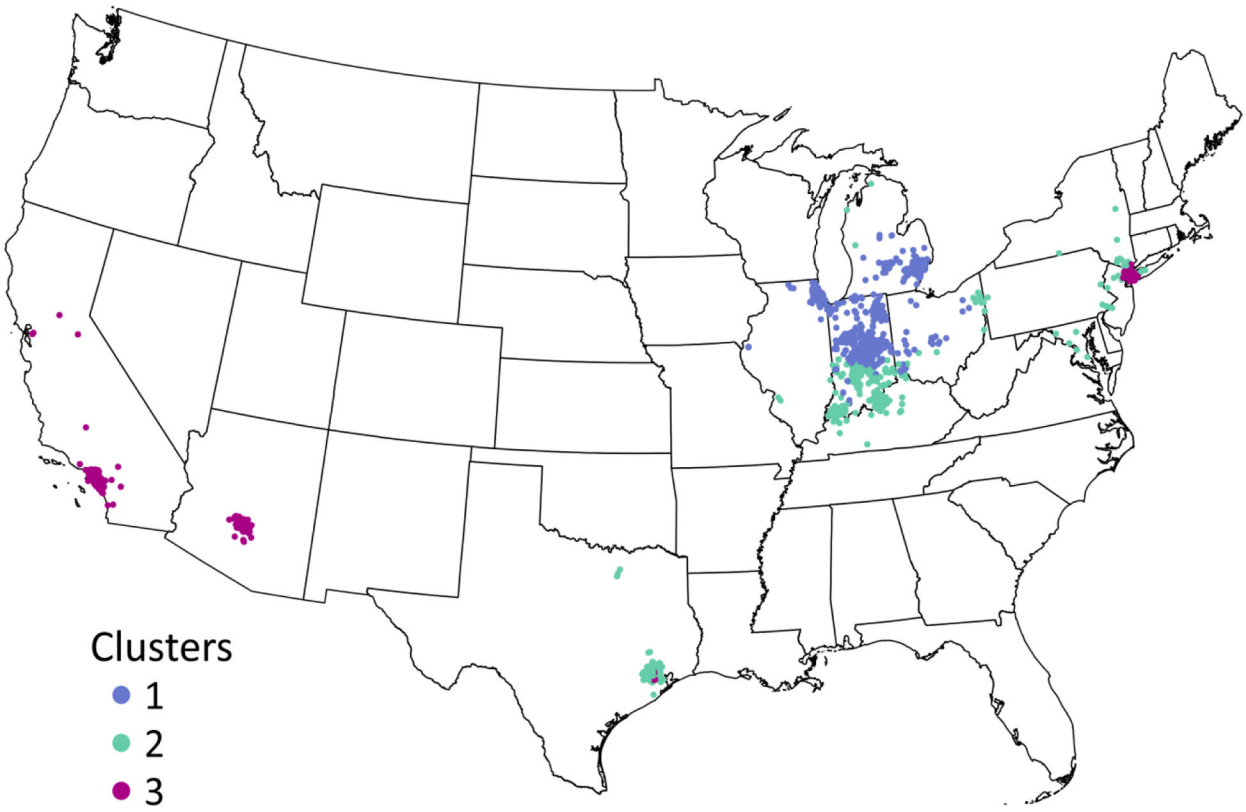

Figure 1.

Map of participant residential locations by $PM_{2.5}$ component cluster group, Komen Tissue Bank, 2009–2019 (N = 3,977). Locations of residences in states in which fewer than 5 participants reside are suppressed.

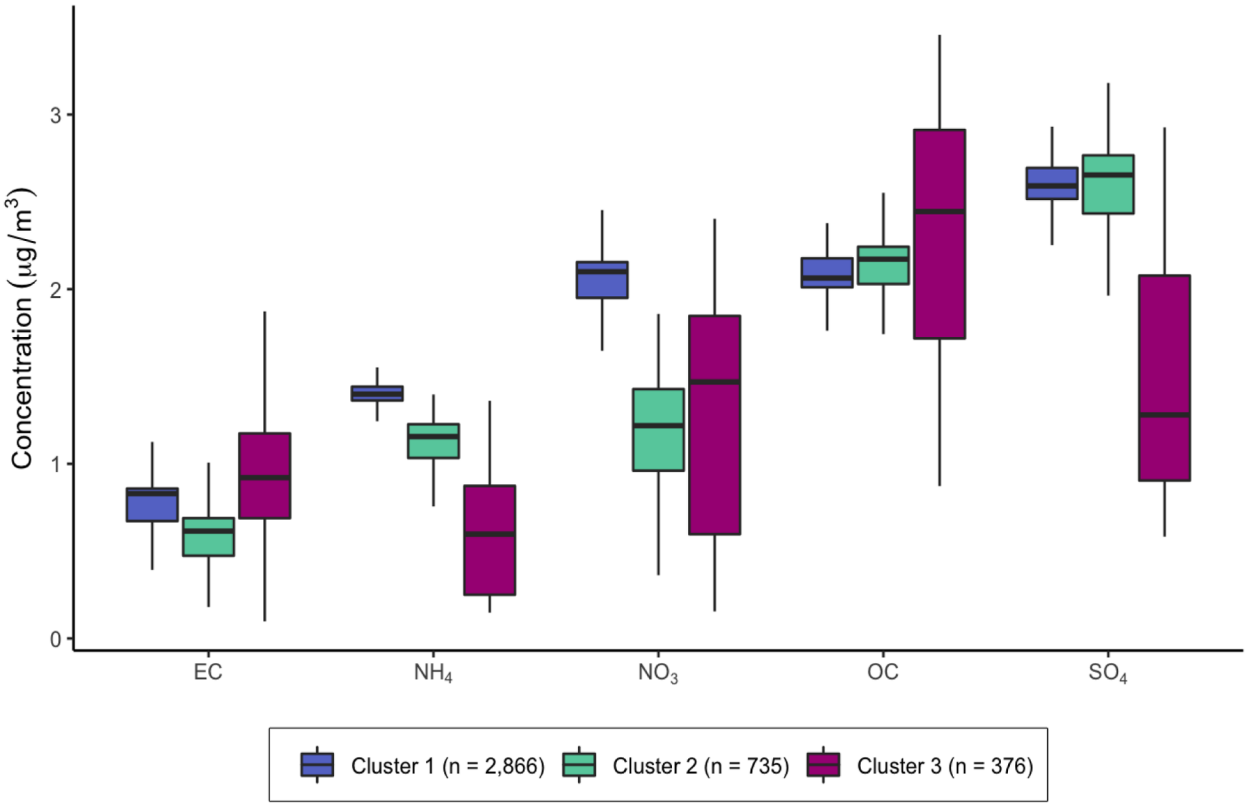

Figure 2.
Distribution of $PM_{2.5}$ component species by cluster group.

Table 1.

Participant characteristics at breast tissue donation, overall and by $PM_{2.5}$ component cluster, Komen Tissue Bank, 2009–2019

	Overall (n = 3,977)	Cluster 1 (n = 2,866)	Cluster 2 (n = 735)	Cluster 3 (n = 376)
Census region, n (%)				
Midwest	3327 (83.7%)	2865 (100.0%)	461 (62.7%)	1 (0.3%)
Northeast	111 (2.8%)	1 (0.0%)	29 (3.9%)	81 (21.5%)
South	260 (6.5%)	0 (0%)	245 (33.3%)	15 (4.0%)
West	279 (7.0%)	0 (0%)	0 (0%)	279 (74.2%)
$PM_{2.5}$(ug/m3)				
Median [IQR]	11.5 [5.6, 14.5]	11.5 [6.8, 14.5]	11.0 [5.6, 14.1]	9.71 [6.1, 12.5]
Age at donation				
Mean (SD)	43.8 (14.2)	43.4 (14.0)	44.4 (14.7)	45.5 (14.5)
Race/ethnicity, n (%)				
Non-Hispanic Black	728 (18.3%)	622 (21.7%)	77 (10.5%)	29 (7.7%)
Non-Hispanic white	2718 (68.3%)	1996 (69.6%)	557 (75.8%)	165 (43.9%)
Hispanic	336 (8.4%)	174 (6.1%)	74 (10.1%)	88 (23.4%)
Other	195 (4.9%)	74 (2.6%)	27 (3.7%)	94 (25.0%)
Education, n (%)				
High school or less	658 (16.5%)	470 (16.4%)	147 (20.0%)	41 (10.9%)
Some college	1001 (25.2%)	680 (23.7%)	218 (29.7%)	103 (27.4%)
Bachelors	1306 (32.8%)	982 (34.3%)	203 (27.6%)	121 (32.2%)
Graduate degree or higher	1012 (25.4%)	734 (25.6%)	167 (22.7%)	111 (29.5%)
Mean (SD)	29.7 (7.7)	29.8 (7.8)	30.0 (7.6)	28.7 (7.2)
< 18.5	44 (1.1%)	26 (0.9%)	10 (1.4%)	8 (2.1%)
18.5 to < 25.0	1208 (30.4%)	874 (30.5%)	198 (26.9%)	136 (36.2%)
25.0 to < 30	1069 (26.9%)	792 (27.6%)	193 (26.3%)	84 (22.3%)
≥ 30	1656 (41.6%)	1174 (41.0%)	334 (45.4%)	148 (39.4%)
Smoking status, n (%)				
Never	2930 (73.7%)	2106 (73.5%)	542 (73.7%)	282 (75.0%)
Former	817 (20.5%)	595 (20.8%)	144 (19.6%)	78 (20.7%)
Current	230 (5.8%)	165 (5.8%)	49 (6.7%)	16 (4.3%)
Menopause status, n (%)				
Premenopausal	2610 (65.6%)	1936 (67.6%)	438 (59.6%)	236 (62.8%)
Postmenopausal	1367 (34.4%)	930 (32.4%)	297 (40.4%)	140 (37.2%)
Urbanicity, n (%)				
Urban	3574 (89.9%)	2651 (92.5%)	550 (74.8%)	373 (99.2%)
Suburban	387 (9.7%)	204 (7.1%)	181 (24.6%)	2 (0.5%)
Rural	16 (0.4%)	11 (0.4%)	4 (0.5%)	1 (0.3%)
Percent fat on the slide, n (%)				
0 to ≤25%	250 (6.3%)	190 (6.6%)	30 (4.1%)	30 (8.0%)

	Overall (n = 3,977)	Cluster 1 (n = 2,866)	Cluster 2 (n = 735)	Cluster 3 (n = 376)
> 25 to ≤50%	502 (12.6%)	393 (13.7%)	67 (9.1%)	42 (11.2%)
> 50 to ≤75%	887 (22.3%)	679 (23.7%)	136 (18.5%)	72 (19.1%)
> 75–100%	2338 (58.8%)	1604 (56.0%)	502 (68.3%)	232 (61.7%)
Year of donation, n (%)				
2009	353 (8.9%)	319 (11.1%)	34 (4.6%)	0 (0%)
2010	360 (9.1%)	329 (11.5%)	31 (4.2%)	0 (0%)
2011	483 (12.1%)	321 (11.2%)	159 (21.6%)	3 (0.8%)
2012	997 (25.1%)	837 (29.2%)	156 (21.2%)	4 (1.1%)
2013	387 (9.7%)	193 (6.7%)	12 (1.6%)	182 (48.4%)
2014	406 (10.2%)	385 (13.4%)	21 (2.9%)	0 (0%)
2015	231 (5.8%)	76 (2.7%)	136 (18.5%)	19 (5.1%)
2016	400 (10.1%)	251 (8.8%)	148 (20.1%)	1 (0.3%)
2017	193 (4.9%)	84 (2.9%)	27 (3.7%)	82 (21.8%)
2018	128 (3.2%)	38 (1.3%)	5 (0.7%)	85 (22.6%)
2019	39 (1.0%)	33 (1.2%)	6 (0.8%)	0 (0%)

Table 2.

Model coefficients and 95% confidence intervals for the change in breast tissue composition (square root-transformed continuous proportions of epithelium, stroma, and adipose, or proportion epithelium relative to epithelium + stroma) per 5-ug/m^3 increase in $PM_{2.5}$

		β (95% Confidence Interval)			
$PM_{2.5}$ total mass[a]	**N**	**Epithelium (%)**	**Stroma (%)**	**Adipose (%)**	**ESP (%)[b]**
Overall	3,977	−0.12 (−0.34, 0.11)	−0.95 (−1.54, −0.35)	0.77 (0.35, 1.19)	0.23 (−0.17, 0.63)
Component Cluster					
Cluster 1	2,866	−0.05 (−0.31, 0.21)	−1.15 (−1.83, −0.46)	0.91 (0.43, 1.39)	0.48 (0.02, 0.94)
Cluster 2	735	−0.14 (−0.47, 0.19)	−1.38 (−2.25, −0.52)	0.99 (0.38, 1.6)	0.04 (−0.54, 0.62)
Cluster 3	376	−0.58 (−0.98, −0.19)	−1.84 (−2.88, −0.8)	1.45 (0.72, 2.19)	−0.32 (−1.02, 0.37)
p for interaction		0.04	0.43	0.38	0.04
Menopause Status					
Premenopausal	2,610	−0.15 (−0.38, 0.09)	−1.11 (−1.74, −0.47)	0.89 (0.45, 1.34)	0.2 (−0.22, 0.62)
Postmenopausal	1,367	−0.1 (−0.36, 0.15)	−0.71 (−1.39, −0.03)	0.59 (0.11, 1.07)	0.24 (−0.21, 0.7)
p for interaction		0.65	0.14	0.11	0.81

Note: ESP, epithelium-to-stroma proportion. Models are adjusted for age at donation, race/ethnicity, education, body mass index (continuous), smoking status, donation year, census region, %adipose tissue area (%ESP models only).

[a] $PM_{2.5}$ total mass concentrations (μg/m^3) estimated by the EPA EQUATES model and assigned according to year of tissue donation

[b] n = 5 missing ESP