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Publication Date

2017

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**Exposure to Ambient Air Pollution and Potential Biological Mechanisms/Biomarkers in
Minority Children with Asthma Living in the United States**

by

Eunice Yujung Lee

A dissertation submitted in partial satisfaction of the
requirements for the degree of
Doctor of Philosophy

in

Environmental Health Sciences

in the

Graduate Division

of the

University of California, Berkeley

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Spring 2017

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ABSTRACT

Exposure to Ambient Air Pollution and Potential Biological Mechanisms/Biomarkers in Minority Children with Asthma Living in the United States

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Rationale: Exposure to ambient air pollution is a major environmental risk factor for chronic diseases such as asthma. Children with asthma can be even more susceptible to the effects of air pollution since their respiratory system is not fully developed and some of the air pollutants can trigger asthma attacks. Over the past decades, scientists and researchers recognized the need to improve our understanding in the biological response mechanisms. The exact underlying mechanisms linking air pollution to disease outcomes, however, are not clear.

Objectives: The overarching aims of this thesis are to investigate the association between exposure to ambient air pollutants and adverse health effects among minority children and identify potential biological pathways from exposure to health end points by considering genetic ancestry and, asthma endotype (atopy) as effect modifiers of the relation between air pollution and telomere length. In Chapter 1, we investigated the association between ambient air pollutants and asthma exacerbations in urban minority children, as well as effect modification by atopy status and African ancestry. In Chapter 2, we conducted a pilot study to gather preliminary information about how telomere length varies in relation to polycyclic aromatic hydrocarbons (PAH) exposure in children living in a highly polluted city. In Chapter 3, we examined the association between ambient air pollutants and telomere length in minority children to understand the potential damage caused by air pollution at the molecular level.

Methods: In Chapter 1, air pollutant exposures were estimated based on residence using U.S. EPA monitoring data and inverse distance weighting. The associations between average daily exposures and asthma exacerbations were estimated by the incident rate ratio (IRR) from a negative binomial regression model. In Chapter 2, we selected asthmatic and non-asthmatic subjects based on their annual average PAH level and described patterns of telomere length, measured by using uniplex polymerase chain reaction (PCR). In Chapter 3, the annual average daily exposure to each of four air pollutants was examined in relation to telomere length.

Results: In chapter 1, exposure to ambient O₃ and NO₂ were associated with asthma exacerbations. Results for PM_{2.5} were null. Exposure-response relationships were linear for O₃ and NO₂ among non-atopic subjects and inconsistent among atopic subjects. Effect modification by African genetic ancestry was present only for O₃; the impact of exposure appeared to be larger for those with higher African ancestry. In chapter 2, we found an inverse linear relationship between PAH and telomere length in a small pilot study. In chapter 3, the association between ambient SO₂ and telomere length was significantly negative, whereas results for PM_{2.5}, NO₂ and O₃ were null.

Conclusions: Our results provide further evidence that exposure to ambient air pollution is a serious environmental risk factor that causes adverse health outcomes among minority children. In addition, the findings suggest that telomere length may be a new biomarker of oxidative stress induced by air pollution. Further studies of genetic ancestry, asthma endotype/phenotype and telomere biology are needed to delineate specific exposure-response mechanisms that can be used to reduce air pollution related disease outcomes.

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INTRODUCTION

Air pollution is a serious global problem because it contributes to global warming, damages the ecosystem and causes adverse health outcomes. As a major environmental risk factor, ambient air pollution is affecting more than 80% of the global population living in urban areas.¹ In the United States, more than half of the population is exposed to air quality levels that do not meet the national standards.² As a result, millions of deaths each year due to stroke, heart disease, lung cancer and chronic and acute respiratory diseases are in part attributable to exposure to air pollution.³⁻⁵ In particular, children and infants are the most vulnerable groups to the effects of air pollution due to their immature respiratory system. For example, many epidemiological studies have shown trends of increasing asthma prevalence in children in both developed and developing countries.^{6,7} Asthmatic children may be even more susceptible to the effects of air pollution since some of the air pollutants can trigger asthma attacks. In order to reduce the incidence and severity of air pollution-related disease outcomes, especially in children and asthmatics, we need to understand biological response mechanisms, the exposure-response relationships, and enforce regulatory standards.

The effects of air pollution have been studied for decades. The London smog episode in 1952 initiated the very first epidemiological study to examine an association between air pollution and mortality.⁸ The excess deaths reported during and after the smog event were linked to coarse particulate matters generated from industrial activities. This particular event increased the public awareness of air pollution and contributed to a significant emission reduction. As a result of such studies and regulations, air quality has improved dramatically in many countries over the past years. However, recent studies have demonstrated that even low air pollution levels were associated with negative health outcomes in both children and adults.^{9,10} In order to eliminate existing problems, scientists and researchers recognized the need to improve our understanding in the biological mechanisms linking air pollution to disease outcomes. This approach can help us to identify and remove the most toxic components of air pollution and provide more targeted and effective ways to protect the individuals who are sensitive to air pollution.

Hence, over the past few decades, animal and cell-based studies were conducted to gather information about toxicological effects associated with different air pollutants and delineate potential mechanisms of pollutant-associated health effects. Within this context, oxidative stress has been identified as the most plausible underlying factor that causes negative health outcomes.¹¹ When pollutant induced free radical containing molecules exceed a certain amount antioxidants that neutralize them, these free radicals oxidize neighboring species. This process leads to oxidative degradation of biologically important molecules, which consequently erodes tissues, protein, lipids and nucleic acids. Oxidative stress can be caused by multiple air pollutants including ozone, NO₂, and particles. For example, ozone is a highly reactive gas, which can cause airway hypersensitivity and pulmonary inflammation by forming free radicals through interacting with substrates present in the lung lining fluids.^{12,13} Therefore, it is important to study the role of oxidative stress and its biomarkers in airway diseases, which can potentially be used for clinical uses including patient diagnosis and therapy.

A number of animal models and clinical studies have demonstrated high correlations between oxidative stress and telomere shortening.^{14,15} Telomeres are multiple copies of hexanucleotides

(TTAGGG) at the end of eukaryotic chromosomes. The high guanine content of the telomeres' sequence makes telomeric DNA especially vulnerable to oxidative stress. Recent air pollution research has shown associations between air pollutants and telomere length.¹⁶ These new findings suggest that telomeres may serve as a new biomarker of oxidative stress induced by air pollution.

In light of this, I investigated the relationships between exposure to multiple air pollutants and asthma outcomes among minority children. I further explored the impact of air pollution at the molecular level by examining telomere length. There are three main chapters in my dissertation. In the first chapter, I hypothesized that exposure to ambient air pollution could increase asthma exacerbation in minority children. In addition, I hypothesized that individuals with atopy and a high proportion of African ancestry may have different response mechanisms due to their underlying biological characteristics. We found that O₃ and NO₂ exposures were positively associated with asthma exacerbations. Our results suggest that individuals with non-atopic asthma might be more susceptible to ambient air pollution exposures due to different inflammation mechanisms between atopic vs. non-atopic asthma. There was an indication that individuals with African ancestry might be more susceptible to ozone exposure. The findings in this study provide valuable information to understand different responses to air pollution exposure due to asthma phenotype related inflammatory mechanisms and genetic susceptibility.

The second chapter describes a small pilot study designed to provide descriptive data on exposure to ambient air pollution and telomere length. The main results in this chapter were consistent with an inverse relationship between polycyclic aromatic hydrocarbons (PAH) and telomere length, and suggested asthmatic participants may have shorter telomeres than non-asthmatics. The results of this study imply that air pollution and asthma may both contribute to telomeric DNA damage. In addition, our pilot study results suggest that telomere length may have potential for use as a biomarker of DNA damage due to environmental exposures and/or chronic inflammation.

In the third chapter, I was able to examine the association between exposure to ambient air pollutants and telomere length in a study with a larger sample size. First, I investigated the associations between multiple air pollutants (O₃, NO₂, PM_{2.5}, SO₂) and telomere length. I then examined effect modification by age to observe if exposure to air pollution had differing effects on telomere length in younger versus older age. We found that exposure to ambient SO₂ was significantly associated with telomere length, whereas exposure to other pollutants was not. This study helped us to better understand how long-term exposure to multiple air pollutants affects telomere length in minority children.

Overall, my dissertation has suggested that exposure to ambient air pollutants has adverse effects among urban minority children. In addition, our findings highlighted the importance of identifying biological pathways, which may be a new novel biomarker of oxidative stress induced by exposure to air pollutants.

PREFACE FOR CHAPTER 1

Ambient air pollution has become the major public health concern in many developed and developing countries due to the increase in emissions from combustion sources. A number of epidemiological studies have shown adverse effects of air pollution. It is possible that certain population groups are more vulnerable to the effects of polluted air including children and people with chronic and pre-existing conditions. For example, studies in the past have shown that exposure to air pollution was associated with increased frequency and severity of asthma outcomes. In this chapter, we examined the relationship between multiple air pollutants and asthma exacerbations after adjusting for confounding factors. We wanted to investigate if exposure to ambient air pollution is associated with asthma symptoms among the minority children. In addition, we used a genetic ancestry marker to examine the gene and environment interaction since race/ethnicity may be related to a high prevalence of asthma and some vulnerable populations are more likely to live close to pollution sources. The information gathered from this study can be used to understand the role of ambient air pollution in chronic disease prevalence in children.

CHAPTER 1:

Asthma Endotype and African Genetic Ancestry Modify the Effect of Ambient Air Pollution on Exacerbations among Minority Children

ABSTRACT

Rationale: In the U.S., asthma disproportionately affects urban minority populations. We have previously demonstrated that African Americans had worse asthma outcomes in response to air pollution when compared to Latinos.

Objectives: The associations between ambient ozone (O₃), nitrogen dioxide (NO₂), and fine particulate matter (PM_{2.5}) exposures and asthma exacerbations in African American and Latino children were assessed. We also investigated whether these associations differed by atopic status and African ancestry.

Methods: Exposures were estimated based on residence using U.S. EPA monitoring data and inverse distance weighting. The associations between average daily exposures and asthma exacerbations over the past 12 months were estimated by the incident rate ratio (IRR) from a negative binomial regression model. Genome-wide African ancestry was estimated in an unsupervised analysis using ADMIXTURE.

Results: The IRR for exacerbation reached 1.92 (95% CI: 1.22-3.03) in the third decile of O₃ exposure and then plateaued. NO₂ was linearly associated with asthma exacerbations with an IRR of 1.03 per ppb (95% CI: 0.99-1.08); results for PM_{2.5} were null. Exposure-response relationships were linear for O₃ and NO₂ among non-atopic subjects and inconsistent among atopic subjects. Effect modification by African genetic ancestry was present only for O₃; the impact of exposure appeared to be larger for those with higher African ancestry.

Conclusions: Our results suggest that exposures to O₃ and NO₂ as risk factors for exacerbation of asthma among minority children. The effect of O₃ differed by asthma endotype. African ancestry appeared to modify asthma exacerbation risk, suggesting that G×E interactions may be population-specific.

1.1. BACKGROUND

Asthma affects 8.6% of the U.S. population under the age of 18 years and is associated with \$56 billion in health care costs and lost revenue.^{1,2} In the U.S., asthma disproportionately impacts urban minority populations; asthma prevalence is 23.5% in Puerto Rican and 13.4% in African American children compared with 7.6% for White children.¹ Asthma exacerbation follows a similar trend and is two- to three-fold higher in African Americans and Puerto Ricans compared to European Americans.³⁻⁵

Exposure to air pollution has been associated with adverse asthma outcomes: increased asthma severity, worse lung function, and higher rates of asthma exacerbations.⁶⁻⁹ Low-income communities and minority groups such as African Americans and Latinos disproportionately live closer to sources of air pollution.^{10,11} We have demonstrated that African American children living in the San Francisco Bay Area are more susceptible to developing asthma from early-life NO₂ exposure than Latino children living in the same neighborhoods, indicating that population-specific risk factors may affect asthma incidence among children exposed to similar amounts of air pollution.¹²

The response to air pollution exposure may vary by asthma phenotype (endotype) because of the different inflammatory mechanisms involved with different phenotypes. For example, asthma exacerbations among atopic patients are mediated through an IgE and eosinophil pathway, which is more relevant to allergen triggers, including dust mites, pollen and mold exposures.¹³⁻¹⁵ In non-atopic asthma, neutrophilic inflammation is predominant; however, what triggers asthma exacerbations through this pathway is not well understood.¹⁶⁻¹⁸ We reason that allergy status modifies associations between exposure to air pollutants and asthma exacerbations.

Latinos and African Americans have varying proportions of African, European, and Native American genetic ancestry, which has important clinical implications. For example, we can improve the diagnosis and prediction of lung function by incorporating genetic measures of ancestry.^{19,20} Therefore, genetic ancestry, a proxy for genetic variation, may play an important role in determining clinical measures and outcomes such as lung growth and asthma severity in response to environmental exposures.²¹ We hypothesize that gene-environment interactions contribute to racial/ethnic differences in asthma morbidity in response to air pollution exposure. Specifically, we reason that the likelihood of pollution-related asthma morbidity may vary by genetic ancestry because some xenobiotic genes are more prevalent among specific populations, which vary by genetic ancestry.

In this study, we examine the relationship between asthma exacerbations and exposure to ambient air pollution during the previous year among minority children living in several metropolitan areas across the United States. In addition, we investigate whether the associations between air pollutant exposures and asthma exacerbation differ by proportions of African ancestry and asthma phenotype, specifically, atopic vs. non-atopic asthma.

1.2.METHODS

1.2.1. Study population

Our study participants are minority children with asthma (ages 8-21) from two concurrent asthma case-control studies, the Genes-environments and Admixture in Latino Americans Study (*GALA II*) and the Study of African Americans, Asthma, Genes & Environments (*SAGE II*). *GALA II* and *SAGE II* were both designed to investigate complex relationships between genetic, social and environmental factors and asthma risk in children living in urban areas in the United States. *GALA II* recruited Latino children with and without asthma from Chicago, New York, Houston, the San Francisco Bay Area and Puerto Rico. *SAGE II* focused on African American children living in the San Francisco Bay Area. To be eligible for *GALA II* and *SAGE II*, participants must have self-identified as Latino or African American and have had four Latino or African American grandparents. In addition, current smokers, participants with a smoking history of at least 10 pack-years, and those who were in the third trimester of pregnancy were not eligible. In this study, we limited our analyses to subjects with asthma in the mainland United States. We excluded Puerto Rican islanders due to limitations in air pollution exposure assessment. All local institutional review boards approved the study and all parents/participants provided appropriate written consent.

1.2.2. Estimation of ambient air pollution exposure

Each participant's exposures to ambient levels of O₃, NO₂, and PM_{2.5} were estimated based on geocoded residential addresses and air quality data from regional monitoring stations. Each address in a participant's residential history was geocoded using TomTom/Tele Atlas EZ-Locate software (TomTom, Amsterdam, the Netherlands). Ambient air pollution data were acquired from the U.S. Environmental Protection Agency Air Quality System. From the recruitment date, we averaged the daily concentrations over the previous 12-month period to estimate annual average exposures by calculating the inverse distance-squared weighted average from the four closest air pollution monitoring stations within 50 km of the geocoded residence. The exposures of those who moved during the course of the year were weighted based on the number of months spent at each residence.

1.2.3. Assessment of genetic ancestry

Participants were genotyped with the Affymetrix Axiom LAT1 array (World Array 4, Affymetrix, Santa Clara, CA), which includes 817,810 SNPs and was specifically designed to capture known genetic variation in Latino and African American populations.²² SNPs which did not pass quality control procedures were removed from the analyses using the following criteria: not meeting platform-specific quality criteria, <95% call rates, and/ or deviation from Hardy-Weinberg equilibrium ($p < 10^{-6}$) within their respective populations (Puerto Rican, Mexican, and Other Latino). This process resulted in 723,888 high-quality autosomal SNP genotypes for the analyses. Subjects were filtered based on 97% call rates, discrepancy between genetic sex and reported gender, cryptic relatedness (identity by descent [IBD>0.3]), and standard Affymetrix Axiom manufacturer's recommendations.

Estimates of genome-wide African, European and Native American ancestries were obtained by means of an unsupervised analysis using ADMIXTURE assuming three ancestral populations.²³ We used reference haplotypes data from European (CEU) and African (YRI) from HapMap phase II, and 95 Native American individuals. Native American reference individuals were genotyped with the Axiom LAT1 array.²⁴ The assessment of ancestry was based on 568,037 SNPs that overlapped among HapMap samples and Native American reference individuals genotyped in this study.

1.2.4. Definition of asthma exacerbations

We used the composite exacerbation score defined by the American Thoracic Society and European Respiratory Society.²⁵ We assigned points for each reported hospitalization (one), emergency department visit (one), and oral steroid use (one point was given to those who used any oral steroid in the last 12 months and an additional point was assigned if the participant used oral steroids for more than two continuous weeks over the 12 months). Points were summed to derive the composite score.

1.2.5. Statistical analysis

The association between annual average daily ambient air pollution exposures and asthma exacerbations over the past 12 months was estimated by the incident rate ratio (IRR) using negative binomial regression. The negative binomial model is an extension of Poisson regression for count data, appropriate here because the variance was greater than the mean. The exacerbation score is not strictly a count of events over the past year because prolonged steroid use was more heavily weighted. Moreover, a large proportion of subjects had a score of zero. Therefore, we also fit a zero inflated negative binomial model and a logistic regression for a binary outcome (any exacerbations over the past year vs. no exacerbation) as a sensitivity analysis.

In a sensitivity analysis, the odds ratios for any exacerbations estimated in logistic regression models were slightly greater than the IRR from the negative binomial models. Since exacerbations were common in this population of children with asthma, and odds ratios tend to overestimate the true risk ratio when the outcome is not rare, we chose to rely primarily on negative binomial regression. We also prefer the main analysis to those from a zero-inflated model because the zero outcomes were likely to have included mild exacerbations that did not end up in emergency department visits or hospitalization. Since all participants were cases with the possibility of exacerbation, there were no structural zeros.

We fit generalized additive models with penalized splines to allow for nonlinearity in the exposure-response relationships. Visual plots of the smoothed curves present the number of exacerbations over the past 12 months on the y-axis, after a transformation back to the original scale, as an exponential function of the annual average daily pollutant exposure. All splines were adjusted for covariates described above.

Baseline characteristics including gender, age, and body mass index (BMI) at the time of recruitment were included in all models. Potential confounding variables were also added if they were associated with both exposure and outcome variables and not on the causal pathway, including exposure to the other pollutants, recruitment season (spring, summer, fall, winter), race/ethnicity (African American, Mexican American, Puerto Rican, Other Latino), study region (San Francisco Bay Area, Chicago, New York, Houston) and composite socioeconomic status (SES). A composite SES score was created based on maternal education, 12-month household income, and insurance level.¹² We categorized the composite SES score into three categories (low, mid and high) based on tertiles of all subjects.

In addition, we stratified the models by atopic status. Individuals with total IgE levels ≥ 100 IU/mL were considered atopic.²⁶

To examine effect modification by genetic ancestry, we first stratified by race/ethnicity. We then treated African ancestry as binary (using the race- or ethnicity-specific median as the cut-off) and stratified the negative binomial regression models, adjusted for age, gender, BMI, maternal education, and study region. Ambient O₃ exposure was categorized in tertiles and NO₂ and PM_{2.5} exposures were treated as continuous variables. Data on maternal education were more complete than for insurance type or 12-month household income. Therefore, we fit models with maternal education (N=1199), rather than the SES score (N=981), to increase power in stratified analyses.

1.3. RESULTS

1.3.1. Study population

Baseline characteristics are shown in Table 1 for subjects who had asthma exacerbation data, as well as air pollution exposure estimates and complete SES information. Demographic characteristics and exposures to air pollutants were similar between those included and not included (S.Table1 and S.Table2). The annual average daily exposures to ambient O₃ were also similar between GALA II and SAGE II subjects (mean \pm sd exposures of 23.2 \pm 5.5 ppb and 21.2 \pm 3.9 ppb, respectively). However, the proportion of subjects with low SES was greater among Latinos than African American participants (S.Figure1.(a)).

1.3.2. Air pollution and asthma exacerbations

Figure 1 illustrates splines from generalized additive models for O₃, NO₂ and PM_{2.5} after adjusting for covariates and confounding variables in all participants. Ozone was not linearly associated with asthma exacerbations. O₃ exposure was therefore categorized into deciles instead of using a continuous scale. Adjusted IRRs for asthma exacerbations in increasing deciles of ambient O₃ exposure are presented in Table 2. The IRR rose in the second decile of O₃ exposure (15.8-18.2 ppb), to 1.58 times the risk of those in the lowest decile (reference) of exposure. Despite the overlap of confidence intervals across the deciles, we did not see evidence of a linear relationship.

By contrast, NO₂ and PM_{2.5} exposures appeared to be linearly related to the outcome and were treated as continuous variables in the negative binomial regression model. In linear models, the IRR increased 1.27 per interquartile range of 8.9 ppb of NO₂ and slightly decreased (IRR=0.98) per ug/m³ of PM_{2.5} exposure. We considered the use of different regression models including logistic, negative binomial, zero-inflated logistic and negative binomial models in sensitivity analyses (S.Table 3).

When stratified by atopic status, the exposure-response trends of ozone differed (Figure 2). Asthma exacerbations increased linearly as O₃ exposures increased in non-atopic subjects whereas the pattern observed among atopic subjects was not linear.

1.3.3. Genetic ancestry and asthma exacerbations

Figure 3 presents ethnicity-specific incidence rate ratios of asthma exacerbations by tertiles of O₃, stratified by proportion of African genetic ancestry (median split for each race/ethnicity).

The distributions of ambient O₃ exposure across race/ethnic groups were similar (S.Figure 1(b)). The interaction between ozone exposure and African ancestry was borderline significant among African Americans (p-value=0.06) (S.Table 5). Although confidence intervals were wide for some effect estimates, the impact of O₃ exposure on asthma exacerbations appeared to be larger among participants whose African ancestry was above the race- or ethnicity-specific median (Figure 3). The results for NO₂ were inconsistent and for PM_{2.5} were null (data not shown).

1.4. DISCUSSION

Urban minority populations have the greatest risk of asthma exacerbations, and within these populations, groups with high African ancestry have the most severe asthma. These same urban minority populations have higher exposures to air pollution, a major risk factor for asthma exacerbations. However, it was unknown whether genetic factors, which differ in allele frequency by ancestry, modify the effect of high levels of air pollution in minority populations. We examined the relationship between annual average daily exposure to air pollution and asthma exacerbations among four racial/ethnic populations of minority children and adolescents with asthma. Both higher levels of O₃ and higher levels of ambient NO₂ were associated with asthma exacerbations. The results for PM_{2.5} were null in the cohort as a whole. The non-linear pollutant-exacerbation association for O₃ was partially explained by atopic status, suggesting different inflammatory pathways may be involved in atopic compared to non-atopic individuals. There was evidence of effect modification by African genetic ancestry among African Americans. These findings are consistent with our hypotheses that air pollution is an important risk factor for asthma exacerbations, and that genetic ancestry and asthma endotype modify the effects of air pollution exposure on asthma outcomes.

1.4.1. Air pollution and asthma exacerbations

Exposures to ambient O₃ and NO₂ were both positively associated with asthma exacerbations after adjusting for baseline characteristics and potential confounding variables, which is

consistent with other studies.²⁷⁻²⁹ Although the elevated rate ratios were statistically significant in several O₃ categories, the exposure-response relationship was not monotonic.

Based on previous studies, we reasoned that non-atopic asthmatic subjects may be more susceptible to air pollution exposures than those with atopy, as they are thought to have more irritant-induced, neutrophilic type asthma. Hence, we conducted further analysis by stratifying on atopic status. The exposure-response relationships appeared to be similar for NO₂ and PM_{2.5} for both atopic and non-atopic subjects. However, we observed different trends of association for O₃; a positive linear trend for non-atopic subjects and a flattened non-linear trend among atopic subjects. It is possible that strong oxidant pollutants such as O₃ can cause inflammation mediated through neutrophil dominant pathways, which contribute to oxidative stress and more severe asthma outcomes for non-atopic asthmatics.^{30,31} For NO₂, a less potent oxidant gas than O₃, we found increasing exposure-response patterns for both atopic and non-atopic participants. NO₂ is known to be able to enhance airway responses to inhaled allergen, which might explain the observed effect in atopic subjects, while an oxidant effect may be operating in non-atopic subjects as we observed for O₃. Our findings highlight the importance of identifying the biological pathways by which different air pollutants may impact asthma.

1.4.2. Effect modification by genetic ancestry

The effects of ambient O₃ exposure on asthma exacerbations differed by the proportion of African genetic ancestry. Additional stratification by race/ethnicity group and low/high African ancestry resulted in wide confidence intervals. However, in all four race/ethnicity groups, those who had more African ancestry appeared more likely to have O₃-related asthma exacerbations than those who had less African ancestry.

African American identity is an independent risk factor for many chronic diseases in the United States.³²⁻³⁴ Previous studies have reported higher morbidity and mortality for asthma, cardiovascular disease and diabetes among African Americans compared to other racial groups.^{1,35,36} Social, environmental and biological factors contribute to racial disparities in disease risk. For example, the expression of systemic oxidative stress may differ by race/ethnicity and potentially by genetic ancestry. African Americans have significantly lower antioxidant enzyme activities, as well as higher inflammatory and oxidative stress markers than other racial groups.³⁷ Hence, the combined effects of O₃ and potentially higher baseline airway oxidative stress/inflammation may have contributed to asthma exacerbations among high African ancestry groups, which may contribute to increased hospitalizations, ER visits, and oral steroid use.

The magnitude and direction of gene by environment interactions differs by racial groups.^{38,39} For example, we have previously observed greater asthma severity and lower lung function among Mexican Americans with a high proportion of European ancestry, compared to those with lower European ancestry.⁴⁰ The effect modification by African ancestry that we observed in the current study may be related to genetic factors. High-risk variants in *APOL1*, a gene associated with chronic kidney disease, are more prevalent in African Americans and the risk allele varies by geography in Africa.⁴¹ It is possible that genome-wide African ancestry may capture genes associated with oxidative stress and/or other risk genes for asthma exacerbations. A common IL-

10 polymorphism present in people with African ancestry has been shown to be protective against helminth infection but may be a risk factor for allergy and asthma in a relatively helminth-free environment.⁴²

While it is possible that genetically determined levels of oxidative stress and inflammation may explain the differential effect modification by African genetic ancestry found in our study, people of African ancestry are also more likely to be exposed to structural discrimination, including living in communities characterized by pollution, poverty, and violence.^{43,44} Therefore, the association between air pollution and asthma exacerbations that we found could have been confounded by unmeasured social factors which were not fully controlled in our analysis. It is challenging to disentangle the causes of multifactorial diseases, but racial discrimination, poor health care access and biological risk factors may act together to increase the risk of asthma exacerbations and therefore asthma morbidity and mortality among African Americans.

1.4.3. Strengths and limitations

One of the major limitations of our study was that the exposure estimations were solely based on the residence of the participants and no time-activity patterns were incorporated into the model. Though exposures may be either under- or over-estimated, the measurement error was not systematic. Moreover, we applied the same air pollution exposure model to all subjects. Therefore, any measurement error was non-differential and would be expected to bias our estimates toward the null.

The composite exacerbation scores may also be subject to measurement error. It is possible that the subjects might have under or over reported their hospitalizations, ED visits, and use of oral steroid medications over the past 12 months. For example, it is likely that they would remember recent events better than exacerbations that occurred many months earlier. Moreover, some mild asthma exacerbations may not have been reported.

Residual confounding by known and unknown socioeconomic factors associated with African ancestry is another limitation of our study; asthma disparities are closely linked to low-income, lack of access to health care, and racial minority status.⁵¹ African ancestry is highly correlated with darker skin color and studies demonstrated that skin color is an important predictor of socioeconomic position.⁵² In addition, low socioeconomic position is associated with residence in areas with high pollution and violence.⁵³ Therefore, socioeconomic factors related to African ancestry need further identification and improved measurement to be better addressed in future studies.

1.5. CONCLUSIONS

We found that exposures to O₃ and NO₂ were positively associated with asthma exacerbations after adjusting for baseline covariates and potential confounding by socioeconomic status. The differing underlying inflammatory mechanisms between atopic vs. non-atopic asthma may make individuals with non-atopic asthma more susceptible to exposures to ambient O₃. Our study also suggests that African genetic ancestry modifies the impact of O₃ on asthma morbidity. We found

evidence that participants with higher African ancestry in each race/ethnicity subgroup had more frequent asthma exacerbations with increasing O₃ exposure than those with lower African genetic ancestry. This apparent effect modification by African ancestry likely reflects a complex interaction between genetic and social factors. Further studies, including admixture mapping and data from whole genome sequencing, are warranted to delineate any specific genetic loci, which may moderate the effects of ancestry on air pollution. These approaches may lead to an improved understanding of those at particular risk, and the possibility of personalized therapy for those at greatest risk in these vulnerable populations.

TABLES

Table 1. Baseline characteristics of SAGE II and GALA II participants (cases).*

Characteristic (N _{total} = 981)	GALA II (N=527)	SAGE II (N= 454)
Age	12.7±3.4	13.6±3.5
BMI [†] (kg/m ²)	24.1±6.7	24.6±7.1
Asthma Exacerbations		
No exacerbation, N (%)	312 (59)	267(58)
Any exacerbation, N (%)	215 (41)	187 (42)
Exacerbations ^{††}	2.01±0.9	2.04±1.0
O ₃ exposure (ppb)	23.2±5.5	21.2±3.9
NO ₂ exposure (ppb)	17.7±6.2	12.7±3.1
PM _{2.5} exposure (µg/m ³)	11.4±1.8	9.5±2.0
African ancestry (%)	10.9±12.4	78.4±12.7
Male, N (%)	281(53.3)	249 (54.8)
Socioeconomic status, N (%)		
Low	362 (68.7)	156 (34.3)
Mid	140 (26.6)	199 (43.8)
High	25 (4.7)	99 (21.8)
Race/ethnicity, N (%)		
African American	.	454 (100)
Mexican American	275 (52.2)	.
Other Latino	177 (33.6)	.
Puerto Rican	75 (14.2)	.
Region, N (%)		
SF Bay Area	91 (17.3)	454 (100)
Houston	102(19.4)	.
Chicago	183 (34.7)	.
New York	151 (28.6)	.

*Values are shown as mean ± SD for continuous variables and frequency and percentage for categorical variables.

[†]BMI was calculated as following, BMI = weight (kg) / (height (m))²

^{††} Among those with any asthma exacerbation

Table 2. Adjusted incident rate ratios for asthma exacerbations by pollutant in a negative binomial model. Incidence rate ratios for asthma exacerbations in relation to annual average daily exposure of ambient O₃ in deciles and NO₂ and PM_{2.5} as continuous variable in all SAGE II and GALA II participants (excluded Puerto Rican Islanders), adjusted in a negative binomial model*.

Variable	N=981	Incident Rate Ratio (95% CI)
Ozone (ppb)		
Categories		
<=15.8	86	1.00
15.8<O ₃ <=18.2	95	1.58 (1.02, 2.48)**
18.2<O ₃ <=19.5	96	1.51 (0.97, 2.37)
19.5<O ₃ <=20.5	92	1.92 (1.22, 3.03)**
20.5<O ₃ <=21.7	94	1.86 (1.17, 2.97)**
21.7<O ₃ <=23.0	107	1.25 (0.76, 2.06)
23.0<O ₃ <=24.0	96	1.55 (0.93, 2.59)
24.0<O ₃ <=25.8	95	1.70 (1.02, 2.87)**
25.8<O ₃ <=28.4	110	1.78 (1.07, 2.98)**
28.4<O ₃ <=43.4	110	1.69 (0.94, 3.04)
NO ₂ (ppb)		1.03 (0.99, 1.08)
PM _{2.5} (µg/m ³)		0.98 (0.92, 1.05)

* Incident rate ratios represent multivariable associations between ozone, NO₂, PM_{2.5} and asthma exacerbations adjusted for age, BMI, gender, composite socioeconomic status (insurance level, 12-month average household income, maternal education level), race/ethnicity, and study region.

** p-value < 0.01

FIGURES

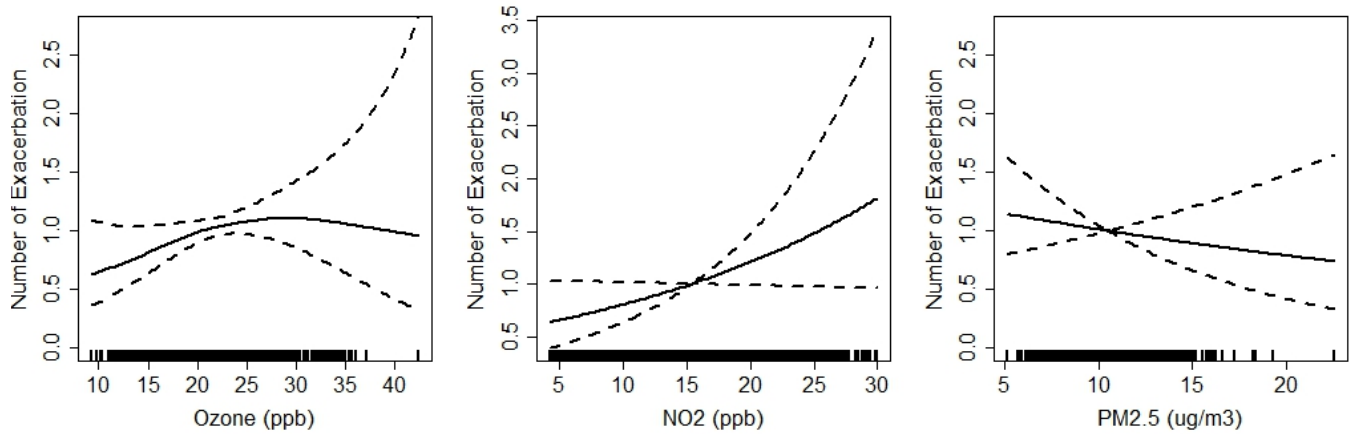


Figure 1. Generalized additive model for exposures to O₃, NO₂ and PM_{2.5} and asthma exacerbations. Generalized additive model fits showing associations between annual average daily exposure to O₃, NO₂, PM_{2.5} and asthma exacerbations. Models adjusted for age, BMI, gender, composite socioeconomic status (insurance level, 12-month average household income, maternal education level), race/ethnicity, study region and co-pollutants.

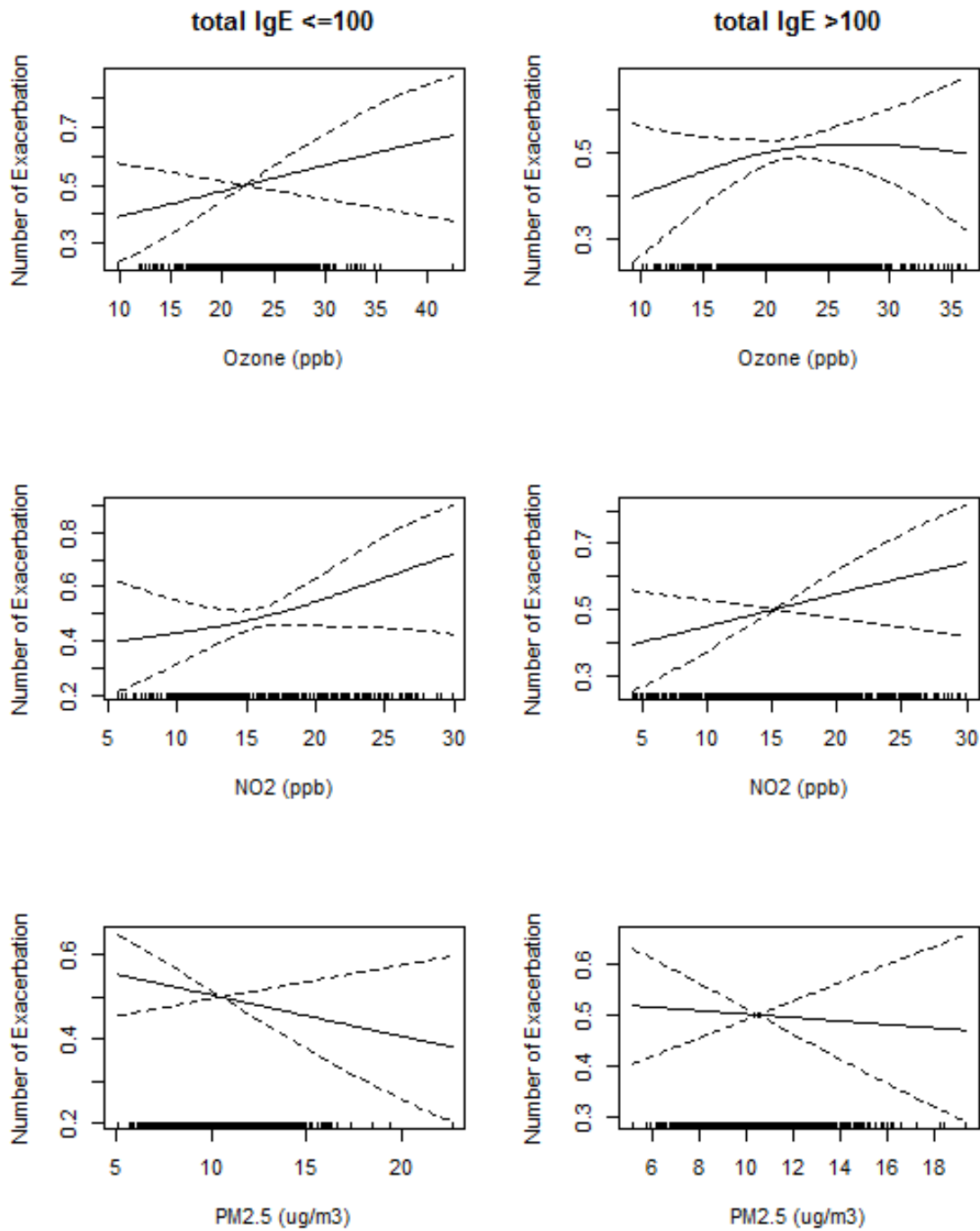


Figure 2. Generalized additive model for exposures to O₃, NO₂ and PM_{2.5} and asthma exacerbation by atopic status. Exposure-response relationships between annual average daily ambient O₃, NO₂, and PM_{2.5} exposure and asthma exacerbations by atopic status. Models adjusted for age, BMI, gender, composite socioeconomic status (insurance level, 12-month average household income, maternal education level), race/ethnicity, study region and co-pollutants.

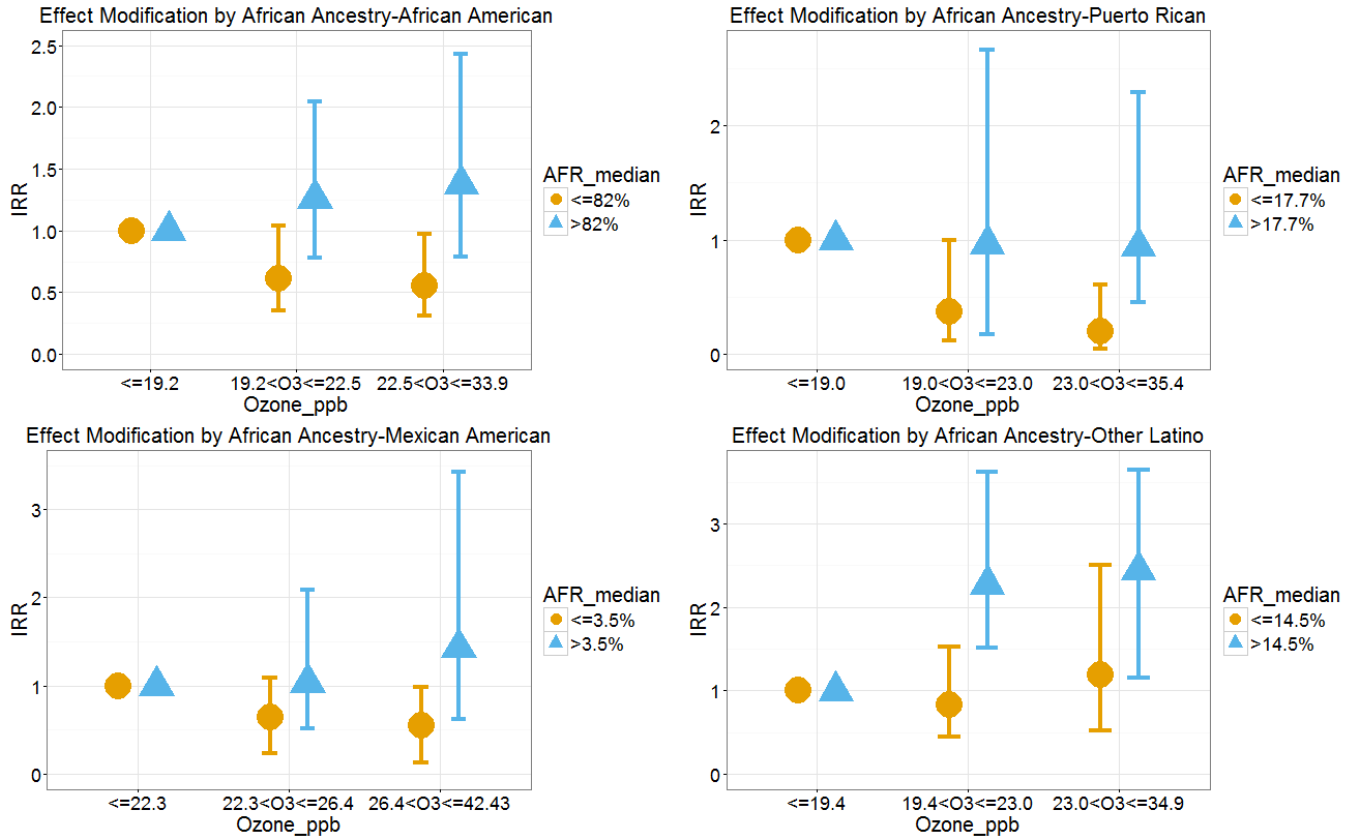


Figure 3. Effect modification of O₃-asthma exacerbation association by genetic ancestry. Data were stratified by race/ethnicity and a median split of African ancestry. Graphs show the incident rate ratio for the association between asthma exacerbation and three levels of annual daily average exposure to ambient O₃. Models were adjusted for age, gender, BMI, maternal education, and study region.

Supplementary Information

S. Table 1. Baseline characteristics of GALA II cases included vs. cases excluded from the study.

Characteristic* (N _{total} =1283)	GALA II_cases excluded (N=756)	GALA II_cases included (N= 527)
Age	12.9±3.2	12.7±3.4
BMI [†] (kg/m ²)	24.6±6.7	24.1±6.7
O ₃ exposure (ppb)	22.1±4.9	23.2±5.5
NO ₂ exposure (ppb)	17.0±5.5	17.7±6.2
PM _{2.5} exposure (µg/m ³)	10.9±2.0	11.4±1.8
African ancestry (%)	10.2±12.1	10.9±12.4
Male, N (%)	422(55.8)	281(53.3)
Race/ethnicity		
Mexican American	437 (57.8)	275 (52.2)
Other Latino	254 (33.6)	177 (33.6)
Puerto Rican	65 (8.6)	75 (14.2)
Region		
SF Bay Area	345 (45.6)	91 (17.3)
Houston	109(14.4)	102(19.4)
Chicago	145 (19.2)	183 (34.7)
New York	157 (20.7)	151 (28.6)

S. Table 2. Baseline characteristics of SAGE II cases included vs. cases excluded from the study.

Characteristic* (N _{total} =988)	SAGE II_cases excluded (N=534)	SAGE II_cases included (N= 454)
Age	14.4±3.7	13.6±3.5
BMI [†] (kg/m ²)	24.8±7.4	24.6±7.1
O ₃ exposure (ppb)	20.9±4.1	21.2±3.9
NO ₂ exposure (ppb)	12.7±3.2	12.7±3.1
PM _{2.5} exposure (µg/m ³)	9.4±2.1	9.5±2.0
African ancestry (%)	80.9±10.9	78.4±12.7
Male, N (%)	256 (48.0)	249 (54.8)
Race/ethnicity		
African American	534 (100)	454 (100)
Region		
SF Bay Area	534 (100)	454 (100)

S. Table 3. Comparison of effect measures for asthma exacerbations in relation to annual average daily exposure to ozone in deciles and NO₂ and PM_{2.5} as continuous variables in all SAGE II and GALA II participants (excluded Puerto Rican Islanders), adjusted in logistic, negative binomial, zero-inflated logistic, and zero-inflated negative binomial regression models*.

Variables	Logistic	Negative Binomial	Zero-inflated	
			Logistic	Negative Binomial
N=981	OR (95% CI)	IRR (95% CI)	OR (95% CI)	IRR (95% CI)
Ozone (ppb)				
<=15.8	1.00	1.00	1.00	1.00
15.8<O ₃ <=18.2	1.70 (0.86, 3.41)	1.58 (1.02, 2.48)**	0.41 (0.15, 1.08)	1.09 (0.69, 1.70)
18.2<O ₃ <=19.5	1.74 (0.87, 3.50)	1.51 (0.97, 2.37)	0.64 (0.26, 1.57)	1.25 (0.81, 1.94)
19.5<O ₃ <=20.5	2.47 (1.24, 5.02)**	1.92 (1.22, 3.03)**	0.51 (0.20, 1.29)	1.40 (0.90, 2.17)
20.5<O ₃ <=21.7	2.34 (1.14, 4.87)**	1.86 (1.17, 2.97)**	0.47 (0.18, 1.22)	1.34 (0.86, 2.09)
21.7<O ₃ <=23.0	1.43 (0.67, 3.10)	1.25 (0.76, 2.06)	0.65 (0.23, 1.84)	1.02 (0.59, 1.74)
23.0<O ₃ <=24.0	2.11 (0.97, 4.62)	1.55 (0.93, 2.59)	0.90 (0.32, 2.51)	1.47 (0.86, 2.50)
24.0<O ₃ <=25.8	2.03 (0.92, 4.52)	1.70 (1.02, 2.87)**	0.68 (0.25, 1.84)	1.46 (0.88, 2.44)
25.8<O ₃ <=28.4	2.16 (0.98, 4.81)	1.78 (1.07, 2.98)**	0.52 (0.18, 1.47)	1.34 (0.81, 2.23)
28.4<O ₃ <=43.4	1.66 (0.66, 4.19)	1.69 (0.94, 3.04)	0.54(0.17, 1.69)	1.29 (0.74, 2.25)
NO ₂ (ppb)	1.06 (0.99, 1.14)	1.03 (0.99, 1.08)	0.99 (0.91, 1.09)	1.03 (0.98, 1.08)
PM _{2.5} (µg/m ³)	0.98 (0.89, 1.09)	0.98 (0.92, 1.05)	0.97 (0.84, 1.12)	0.97 (0.91, 1.04)

* Model includes age, BMI, gender, composite socioeconomic status (insurance level, 12-month average household income, and maternal education level), race/ethnicity, and study region.

** p-value < 0.01

S.Table 4. Relationship between covariates and asthma exacerbation in SAGE II and GALA II patients.

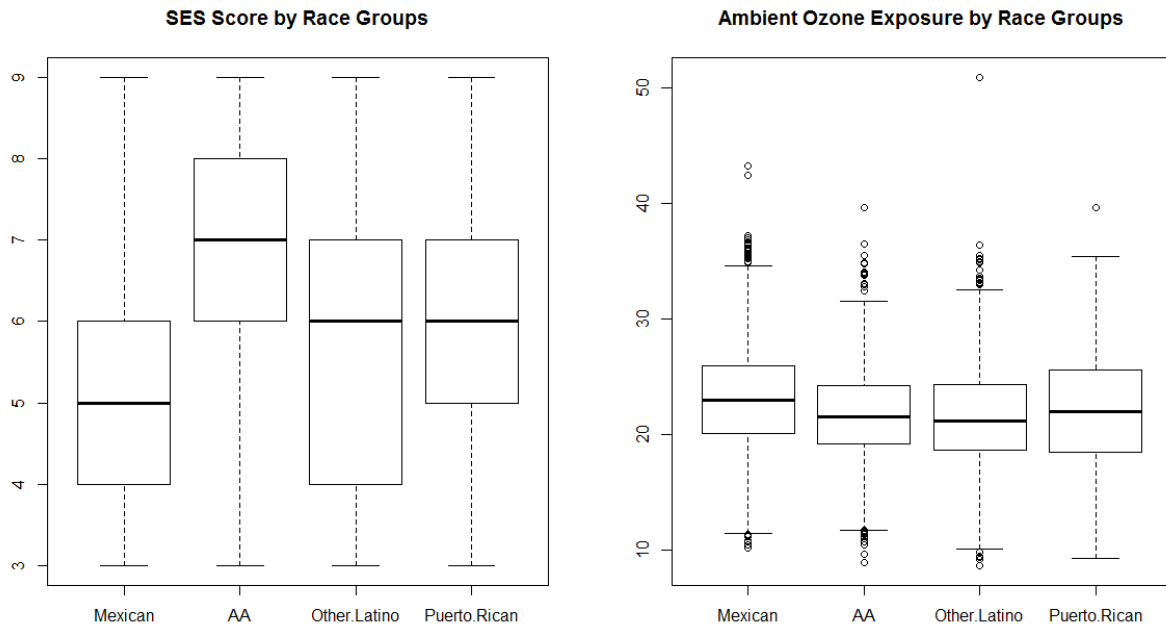
Variable Name	N=981	Incident Rate Ratio (95% CI)
Age – yr		0.94 (0.91, 0.96)
SES composite categories, N (%)		
High	124	1.00
Mid	339	1.26 (0.91, 1.77)
Low	518	1.42 (1.02, 1.99)
Race/ethnicity, N (%)		
Mexican American	275	1.00
African American	454	1.55 (1.05, 2.30)
Other Latino	177	1.58 (1.14, 2.20)
Puerto Rican	75	2.07 (1.35, 3.16)
Region		
Chicago	183	1.00
New York	151	1.58 (1.05, 2.39)
San Francisco	545	1.86 (1.05, 3.34)
Houston	102	2.28 (1.25, 4.15)

S. Table 5. Two-way interaction between ozone and African ancestry. Interaction between ambient ozone exposure as a continuous variable and African ancestry as a binary (greater or less than the median split for each race/ethnicity) in SAGE II and GALA II patients, adjusted in a negative binomial model* .

Ethnicity/race group	N=981	Incident Rate Ratio (95% CI)
African American	454	1.07 (1.00, 1.15) **
Mexican American	275	1.03 (0.96, 1.12)
Other Latino	177	0.98 (0.92, 1.05)
Puerto Rican	75	1.02 (0.95, 1.09)

*Model includes age, BMI, gender, maternal education level, and study region.

** p-value <0.05



S.Figure 1. (a) Distribution of the composite SES score by race/ethnicity; (b) Boxplots representing the distribution of annual average daily ambient ozone exposure by race/ethnicity.

Generalized Additive Model (GAM)

GAM is an unspecified non-parametric function uses a cubic smoothing spline to find the trend of dependent variable Y as a function of one or more independent variables X_1, \dots, X_p ,

$$Y = \alpha + \sum_{z=1}^p \beta_z (X_z) + \sum_{j=1}^p f_j(X_j) + \varepsilon \quad (1)$$

PREFACE FOR CHAPTER 2:

In the previous study, we investigated the relationship between ambient air pollution and asthma exacerbations in minority children after adjusting for confounding factors. However, the associations between different air pollutants and asthma exacerbations were inconsistent. Part of these inconsistencies was explained by different underlying inflammatory mechanisms related with atopy status. In addition, it is possible that the interaction between genetic and social factors may have modified the effect of air pollution on asthma exacerbation. One of the main limitations of the study was that it is not clear how exposure to air pollution led to the asthma outcome at the molecular level. We aimed to begin to address these issues in this chapter. In an attempt to better understand the underlying biological mechanism from air pollution exposure to health endpoint, we considered telomere length as a bio-marker of oxidative stress. We chose to conduct a small pilot study of telomere length because of its unique ability to control the life span of important cells in our body, which may contribute to health outcomes. We hypothesized that long-term exposure to air pollution affects telomere length, which in turn increases susceptibility to adverse health effects.

CHAPTER 2:

Traffic-Related Air Pollution and Telomere Length in Children and Adolescents Living in Fresno, CA: A Pilot Study

ABSTRACT

Rationale: Overwhelming evidence demonstrates that exposure to air pollution is associated with adverse cardiopulmonary outcomes, however, the underlying mechanisms are not yet clear. One plausible pathway is via reactive oxygen species generated during incomplete combustion of fossil-fuels that can potentially lead to tissue injury and inflammation. To better understand the effects of ambient air pollution at the molecular level, we designed a pilot study of polycyclic aromatic hydrocarbons (PAH) and telomere length (TL) in a group of children living in Fresno California.

Objective: The main objective of this pilot study was to gather preliminary information about how telomere length (TL) varies in relation to polycyclic aromatic hydrocarbons (PAH) exposure in an ethnically diverse group of children living in a highly polluted city.

Methods: We conducted a cross-sectional study of children living in Fresno, California (n=14). Subjects with and without asthma were selected based on their annual average PAH level in the 12-months prior to their blood draw. We measured relative telomere length from peripheral blood mononuclear cells (PBMC).

Results: We found an inverse linear relationship between PAH and telomere length (TL) ($R^2 = 0.69$), as well as between age and TL ($R^2 = 0.21$). Asthmatics had shorter mean telomere length than non-asthmatics ($TL_{\text{female}}=1.13$, $TL_{\text{male}}=1.29$).

Conclusions: These preliminary findings suggest that exposure to ambient PAH may play a role in telomere shortening.

2.1. BACKGROUND

In many urban settings, ambient air pollution is a major public health concern because of the associated burden of disease. According to the World Health Organization, outdoor air pollution is responsible for about 3.7 million deaths annually on a global basis (1). In the United States, exposure to traffic-related PM_{2.5} (particulate diameter ≤ 2.5 μm) may contribute to as much as 20% of total mortality (2). Air pollutants also appear to play an important role in the onset of many chronic diseases including asthma, lung cancer, ischemic heart disease and stroke (3–6). A number of epidemiological studies have demonstrated that exposures to particulate matter and ozone were associated with increases in cardiopulmonary mortality (7,8). Despite this mounting evidence, the exact underlying mechanisms by which air pollutants cause adverse cardiopulmonary health outcomes are not clear.

Animal studies have suggested several biological mechanisms to explain how air pollution induces disease outcomes (9). One possible mechanism is that the free radicals generated during the incomplete combustion of fossil-fuel products cause oxidative stress within the respiratory and cardiovascular systems (10). Oxidative stress occurs when free radicals exceed the relative amount of antioxidants. Reactive oxygen species (ROS), a common class of free radical, are generated with inhalation of certain air pollutants. Evidence from a number of epidemiological studies indicates that air pollution causes oxidative stress, which is capable of damaging lipids, proteins, and DNA (10–12). Since telomeres play a critical role in chromosome stability and cell viability, it is reasonable to use telomere length as a biomarker for air pollution induced cytotoxicity.

Recent studies of telomere length and exposure to high levels of traffic-related air pollutants in healthy adults have found shortening of telomeres associated with increasing air pollution levels (13,14). Telomeres are multiple short sequences of DNA located at the end of linear eukaryotic chromosomes (5'AGGGTT2') (15). Maintenance of telomere length is important for cell viability because cells with short telomeres lose their ability to divide and become senescent or undergo apoptosis (16). In addition, telomeres protect chromosomes against inappropriate recombination and fusion with other broken chromosomes, which can potentially lead to malfunction, cancer, or cell death (15,16). Since the guanine base is more prone to be oxidized than other DNA bases, the high guanine content of the telomere sequence makes telomeric DNA vulnerable to oxidative stress (17,18).

Children may be especially vulnerable to the effects of telomeric DNA damage due to their physical development as well as developing immune system. One study has shown different telomere attrition rates among newborns, their parents, and grandparents (19). This suggests that children may have different telomere regulation than adults and thus may be differentially susceptible to effects of air pollution.

As the first step towards a better understanding of the long-term health effects of traffic-related air pollution on telomere length, we conducted a pilot study to gather information about how telomere length varies in relation to air pollution, age, sex, and asthma status. In this study, we focus on polycyclic aromatic hydrocarbons (PAHs). PAHs are a class of different chemical compounds characterized by fused benzenoid aromatic rings (20). PAHs are produced during

incomplete combustion of organic matter. They exist in ambient air in both gas- and particle-phases (adsorbed to particulate matter). In many urban environments, motor vehicle exhaust is the main source of high-molecular-weight PAHs (four to six rings), which are more carcinogenic and mutagenic than low-molecular-weight PAHs (two- and three-rings) (21). Reported ambient levels of PAHs ranged between 0.02-1.2 ng/m³ in rural areas, and 0.15- 32.9 ng/m³ in urban environments in the United States (22-24). PAHs are ubiquitous ambient air pollutants in Fresno and can be transformed into quinones in the atmosphere (25–27). Quinones can serve as catalysts in redox cycling and generate free radicals (25,26).

2.2. METHODS

All methods and procedures were approved by the institutional review boards of Stanford University and the University of California, Berkeley.

2.2.1. Study subjects

Subjects were selected from a larger population of children enrolled in an ongoing study of asthma in Fresno, CA (Figure1). They were age 11 to 18 years old, living in Fresno, California. Fresno is located in the center of the San Joaquin Valley, which is part of the Central Valley in California. Fresno is the second-most polluted city in the United States, in terms of 24-hour average PM_{2.5} (28) and has a high prevalence of asthma (29). For the pilot study, 14 subjects were selected from high- and low-exposure groups, as defined by annual average 24-hour outdoor residential exposure to PAHs in the 12 months prior to their blood draw (2009-2012). The high-exposure group was defined as above the 80th percentile of PAH exposures and the low-exposure group below the 10th percentile. An equal number of subjects (n=7) were selected from the high- and low-exposure groups.

Study participants came from two related studies, the initial Fresno Asthmatic Children's Environment Study (FACES), and the subsequent Children's Health and Air Pollution Study (CHAPS). FACES was a longitudinal cohort study designed to follow children with asthma. CHAPS focused on the health risks of air pollution exposure in both asthmatic and non-asthmatic children in the San Joaquin Valley. Of the 14 subjects in the pilot, 5 were asthmatic, originally recruited for FACES, and 9 non-asthmatic subjects were recruited for CHAPS. At the baseline interview, all subjects provided detailed information on their general history and respiratory health. FACES study participants had asthma and underwent pre- and post-bronchodilator spirometry and skin prick testing for 14 aeroallergens common in the Fresno area. CHAPS subjects were defined as non-asthmatic and non-allergic if they had (1) no reported physician diagnosis of asthma, (2) normal pulmonary function test results, (3) total IgE (immunoglobulin E) <10IU/mL, and (4) negative skin test results. Further details on the study design and cohort characteristics can be found in papers published elsewhere (30–33).

2.2.2. Individual PAH exposure estimates

To estimate the daily individual exposures to ambient PAHs, we developed a land use-regression model using PAH measurements from both a central monitoring site and outside of a subset of

FACES participants' homes. Outdoor concentrations of PAHs with three rings or greater were measured. The filter-based PAH samples provided concentrations for 14 PAHs. However, we chose to use PAHs with 4-, 5- or 6-rings as a metric representing the less volatile, particle-bound PAHs. This selected group of PAHs (PAH456) had a good correlation with the continuous measure of PAHs we were using in the spatial-temporal model. The measured PAH data for the sum of PAHs with 4-, 5-, and 6-six rings (PAH456) were used as a dependent variable of a regression model with a large number of independent land use variables. Good agreement between predicted and measured concentrations of PAH456 was reported with the final model. The model parameters were then used to calculate individual daily exposure to ambient PAH456. More information on the model selection/parameters and field sampling of PAH456 can be found in a previously published paper (27).

2.2.3. Telomere length measurement

Total genomic DNA was purified from peripheral blood mononuclear cells (PBMCs) using QIAamp® DNA Mini kit (QIAGEN, Cat#51104). The telomere length assay was adapted from the published original method by Cawthon (34,35). Telomere length was determined by relative ratio of telomere gene copy number to single copy gene copy number in each sample to reference DNA sample. The telomere thermal cycling profile consisted of:

Cycling for T(telomic) PCR: denature at 96°C for 1 second, anneal/extend at 54°C for 60 seconds, with fluorescence data collection, 30 cycles. Cycling for S (single copy gene) PCR: denature at 95°C for 15 seconds, anneal at 58°C for 1 second, extend at 72°C for 20 seconds, 8 cycles; followed by denature at 96°C for 1 second, anneal at 58°C for 1 second, extend at 72°C for 20 seconds, hold at 83°C for 5 seconds with data collection, 35 cycles.

The primers for the telomere PCR were *tel1b* [5'-CGGTTT(GTTTGG)₅GTT-3'], used at a final concentration of 100 nM, and *tel2b* [5'-GGCTTG(CCTTAC)₅CCT-3'], used at a final concentration of 900 nM. The primers for the single-copy gene (human beta-globin) PCR were *hbg1* [5' GCTTCTGACACAACACTGTGTTCACCTAGC-3'], used at a final concentration of 300 nM, and *hbg2* [5'-CACCAACTTCATCCACGTTTACC-3'], used at a final concentration of 700 nM. The final reaction mix contained 20 mM Tris-HCl, pH 8.4; 50 mM KCl; 200 μM each dNTP; 1% DMSO; 0.4x Syber Green I; 22 ng E. coli DNA per reaction; 0.4 Units of Platinum Taq DNA polymerase (Invitrogen Inc.) per 11 microliter reaction; 0.5 - 10 ng of genomic DNA. Tubes containing 26, 8.75, 2.9, 0.97, 0.324 and 0.108ng of a reference DNA (from HeLa cancer cells) were included in each PCR run so that the quantity of targeted templates in each research sample can be determined relative to the reference DNA sample by the standard curve method. The same reference DNA was used for all PCR runs.

To control for inter-assay variability, eight control DNA samples were included in each run. In each batch, the the ratio of telomere to single copy gene (T/S) of each control DNA was divided by the average T/S for the same DNA from 10 runs to get a normalizing factor. This was done for all eight samples and the average normalizing factor for these samples was used to correct the participant DNA samples to get the final T/S ratio. The T/S ratio for each sample was measured twice. When the duplicate T/S value and the initial value varied by more than 7%, the sample

was run a third time and the two closest values were reported. The coefficient of variation (CV) for this study was typically 2.5%.

2.2.4. Statistical analysis

Linear regression was used to estimate the association between PAH456 and TL, adjusting for age, sex, race/ethnicity (Latino and White) and asthma status. In a sensitivity analysis, the oldest subject with the lowest TL was excluded.

2.3. RESULTS

Table 1 displays the summary characteristics of study subjects. The mean age, telomere length and PAH456 exposure are presented by sex, race/ethnicity and asthma status in Table 2. On average, TL was shorter in the higher PAH456 group; the difference in relative telomere length between the lowest and highest PAH456-exposed individual participants was 0.36.

Crude regression models for TL on age (Figure 2) and PAH456 (Figure 3) suggest inverse linear relationships for both. In a multivariable regression model, telomere length (TL) decreased by -0.14 units (95% CI: -0.25,-0.11) per one ng/m^3 increase in PAH456, adjusting for age, sex, race/ethnicity and asthma (Table 3). Altogether the covariates explained 83% of the variance in TL. Female participants had slightly longer mean telomeres than males ($\text{TL}_{\text{female}}=1.25$, $\text{TL}_{\text{male}}=1.21$). Asthmatic participants had shorter mean telomere length than non-asthmatic participants ($\text{TL}_{\text{asthmatic}}=1.13$, $\text{TL}_{\text{nonasthmatic}}=1.29$). The shortest telomere length ($\text{TL}=0.96$) was found in the subject with the highest PAH456 exposure ($4.2 \text{ ng}/\text{m}^3$). This subject was a 17 year-old Caucasian male asthmatic participant and his TL was between 1 and 2 standard deviations below the mean. After excluding this participant in sensitivity analysis, the association with PAH456 remained significant and the model R^2 decreased to 72%.

Asthmatic participants were exposed to higher levels of PAH456 than non-asthmatic participants (Figure 4). There were more male asthmatic participants in our sample than females and male participants were exposed to a wider range of PAH456 levels (Figure 5).

2.4. DISCUSSION

To the best of our knowledge, this is the first study to investigate the relationships between traffic-related air pollution, specifically ambient PAHs, and telomere length in children in the United States. We found that telomere length decreased with increasing PAH exposure among the small group of participants in this pilot study, consistent with the hypothesis that PAH exposure may cause oxidative stress that can accelerate telomere shortening. The fit of a linear model for TL and exposure to ambient PAH456 improved when adjusted for age, sex, race/ethnicity and asthma status. Therefore, our results also suggest that age, sex, and asthma status may influence the length of telomeres in children.

2.4.1. Air Pollution and Telomere Length

The relationship between PAH exposure and telomere length we observed in this study of adolescents is consistent with studies in healthy adults that have shown telomere shortening with increasing air pollution levels (13,14,36–38). For example, Hoxha et al. reported mean leukocyte telomere length (LTL) among traffic officers in Milan, Italy was 1.10 (95% CI: 1.04-1.16) compared to a mean LTL in office workers of 1.27 (95% CI: 1.20-1.35) (14). In our younger participants, the mean telomere length of the subjects with the lowest PAH exposure was 1.38, whereas the telomere length of the participant with the highest PAH exposure was 0.96. Previous studies have reported a dose-response relationship between PAH exposure and biomarkers of oxidative stress (39,40). Although preliminary pilot data, our results are consistent with the hypothesis that exposure to ambient PAHs (largely generated during combustion of diesel and gasoline fuels in Fresno) leads to oxidative stress, which in turn causes telomere shortening.

2.4.2. Age and Telomere Length

Multiple studies have reported a trend of decreasing telomere length with increasing age (36-38). Most cells, with the exception of some germline and stem cells, lose their telomerase activity once they are differentiated into specific tissue or blood cells (36). In addition, there is less production of stem cells and other renewing cells with increasing age (41). In our participants, we found a weak inverse relationship between age and telomere length which could be due to the narrow age range of the subjects, or different telomere regulation in children and adolescents than that in newborns or adults. Previous studies have shown different telomere lengths and rates of telomere sequence loss with different age groups(19,36,37). Newborns had the most rapid loss of telomeres. The changes in telomere length in later life are rather gradual with advancing age. The longer telomere lengths in newborns reflect a large proportion of immature hematopoietic progenitors that have not gone through extensive proliferation relative to adults (36,41).

2.4.3. Sex and Telomere Length

Female participants had slightly longer telomeres than male participants, consistent with other studies (42,43). In a meta-analysis of telomere length by sex from 36 cohorts (n=36,230), females had longer telomeres than males. Several theories have been proposed to explain telomere length difference by sex. One is related to an estrogen-responsive element that can stimulate telomerase, an enzyme that synthesizes telomere sequences and adds them to the end of chromosomes (43). Another theory is that the properties of estrogen can counteract oxidative stress by up-regulating antioxidant enzyme expression (44). Another alternative explanation for the sex difference between females and males in this pilot study may be that there were more male than female participants with asthma.

2.4.4. Asthma and Telomere Length

Asthma is a chronic inflammatory disease in the airways characterized by recurring exacerbations (45). Frequent inflammatory responses and rapid cell proliferation can lead to telomere shortening (46,47). Exposure to high levels of air pollution can trigger exacerbations of

asthma that could lead to telomere shortening (48-50). Although the annual average concentration of ambient PAHs was higher among the asthmatic compared to the non-asthmatic participants, it is not possible in this pilot study to make inferences about whether the shorter telomeres in asthmatic children were due to their condition or due to exposure to high levels of PAH, or both.

2.4.5. Strengths and Limitations

Previous studies have reported shorter telomere length in children in relation to community stress, poverty, and social deprivation (51), but as noted above, ours is the first to address air pollution. Additional strengths of our study include a novel marker of traffic-related air pollution, PAHs, and a novel biomarker of air pollution-related cytotoxicity, telomere length. Another is our focus on children for whom relatively scant data are available on the association between air pollution and telomere length.

There are several limitations of this pilot study. The primary limitation is the small sample size. Another major limitation is that the cross-sectional design limits the ability to make temporal inferences about whether telomere length shortening occurred after exposure to air pollution.

2.5. CONCLUSIONS

Our pilot study results suggest that telomere shortening in children may be associated with exposure to traffic-related air pollution. Greater knowledge of the impact of air pollution at the molecular level is necessary to design effective interventions and policies. Our preliminary data will inform the design of a larger study to examine the hypothesis generated from these results.

TABLES

Variable	Mean	SD	Range
Age (years)	14.0	2.11	11-18
Telomere length (a.u.)	1.23	0.13	0.96-1.43
PAHs exposure (ng/m ³)	2.98	0.58	2.1-4.2
	%		
Female	50		
Asthmatic	36		
Latino	36		

Table 1. Summary characteristics of Fresno pilot study subjects (n=14)

Subgroup	n	Age (yrs)	TL (a.u.)	PAHs (ng/m ³)
Gender				
Male	7	13.3	1.21	2.88
Female	7	14.7	1.25	2.97
Ethnicity				
Latino	9	13.8	1.20	3.07
White	5	14.4	1.28	2.68
Asthma				
Asthmatics	5	14.4	1.13	3.22
Non-asthmatics	9	13.8	1.29	2.77

Table 2. Mean age, telomere length and PAHs exposure by subgroups

Predictor	Coef	St.Error	t-value	Pr(>t)
(Intercept)	1.80	0.15	12.00	<.01
PAH (ng/m ³)	-0.14	0.04	-3.50	0.01
Age (yr)	-0.0086	0.013	-0.66	0.54
Gender	-0.04	0.05	-0.80	0.46
(ref group: male)				
Race/ethnicity	0.01	0.05	0.20	0.79
(ref group: white)				
Asthma status	-0.07	0.05	-1.40	0.19
(ref group: asthmatic)				

Table 3. Multivariable linear regression to predict telomere length (n=14)

Residual standard error: 0.066 on 8 degrees of freedom
 Multiple R-squared: 0.83, Adjusted R-squared: 0.72
 F-statistic: 7.849 on 5 and 8 DF, p-value: 0.0059

FIGURES

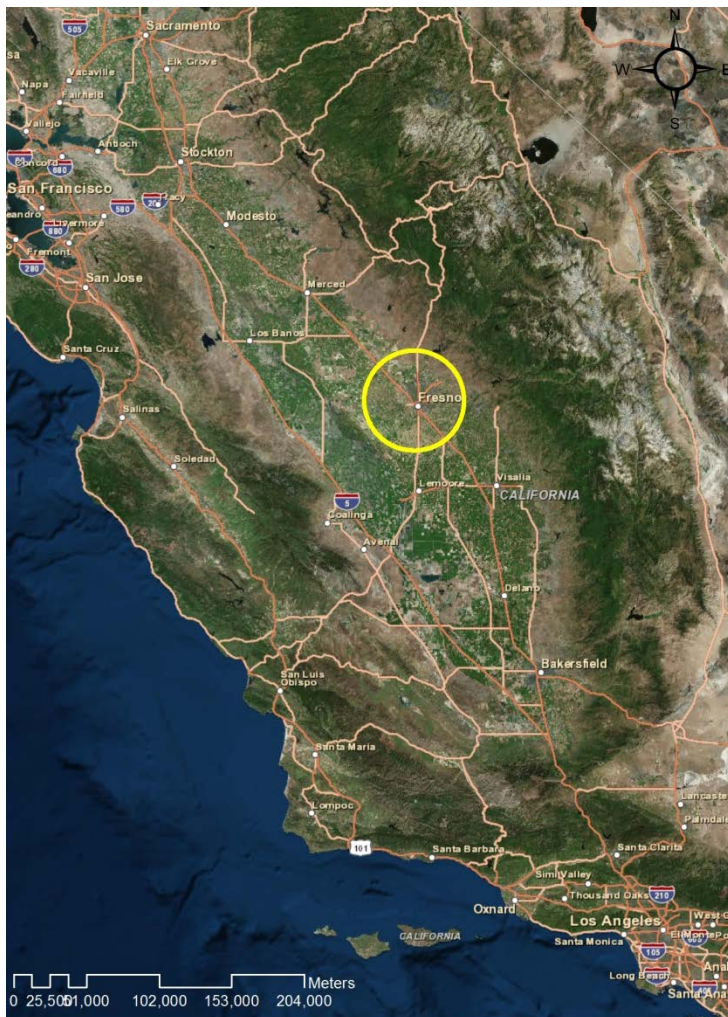


Figure 1. Study subjects were recruited from the Fresno, CA. Fresno is located in the center of the San Joaquin Valley, which is part of the Central Valley in California.

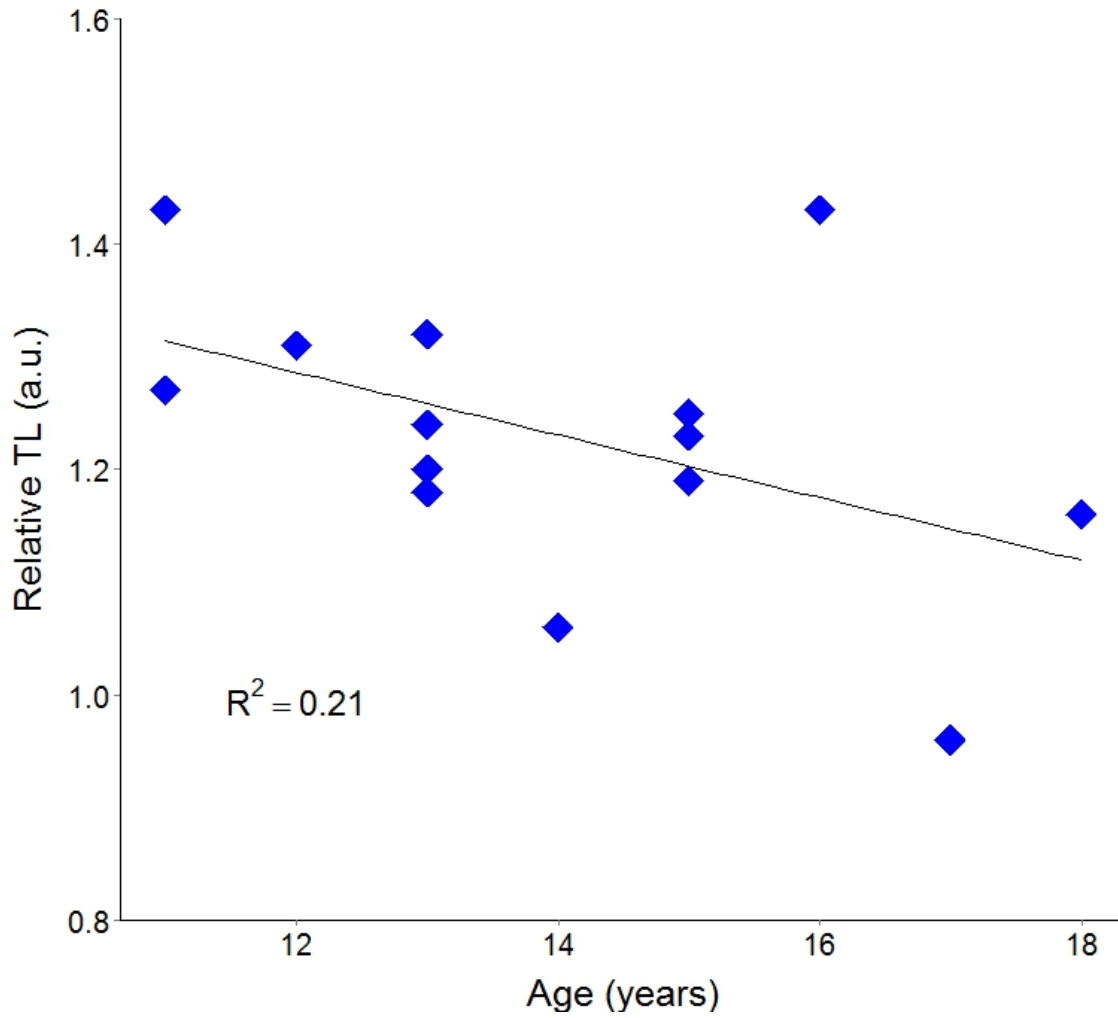


Figure 2. Scatter plot showing the crude linear relationship between age and relative telomere length.

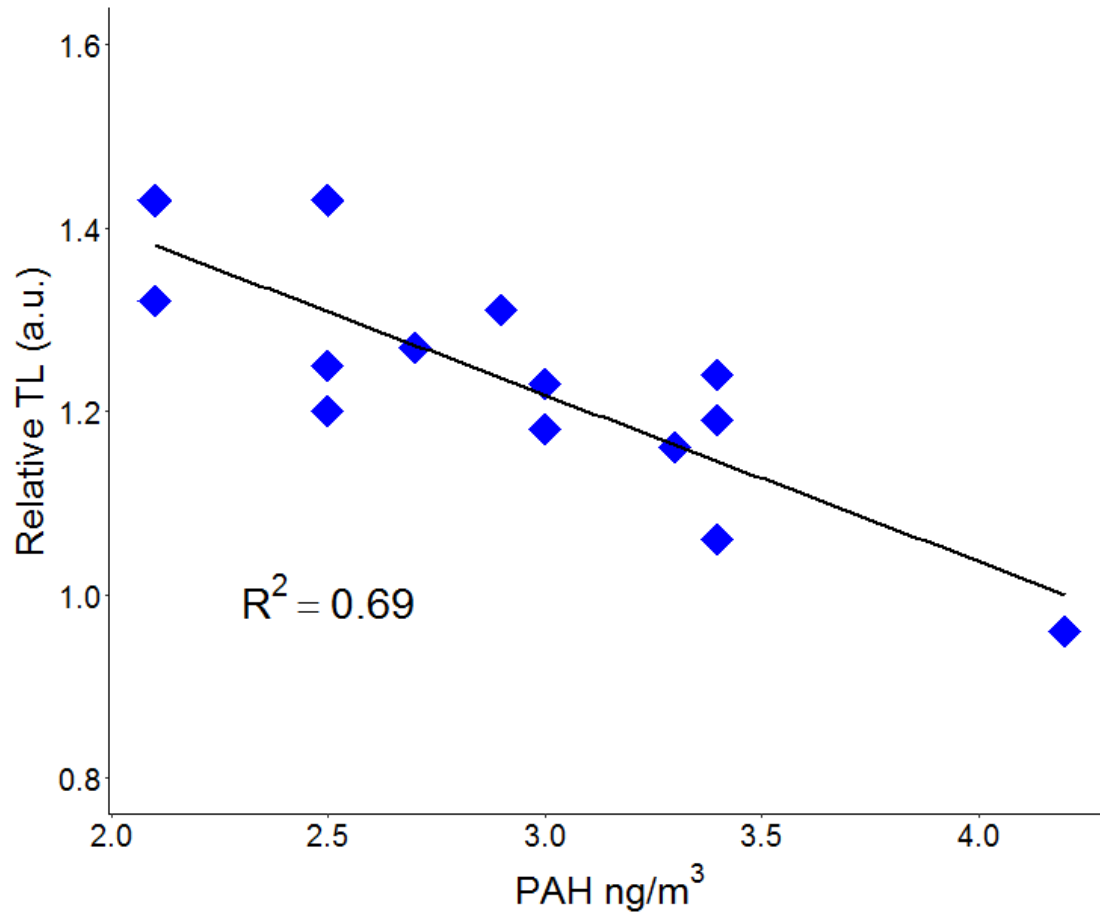


Figure 3. Scatter plot illustrating the crude linear relationship between PAH exposure and relative telomere length.

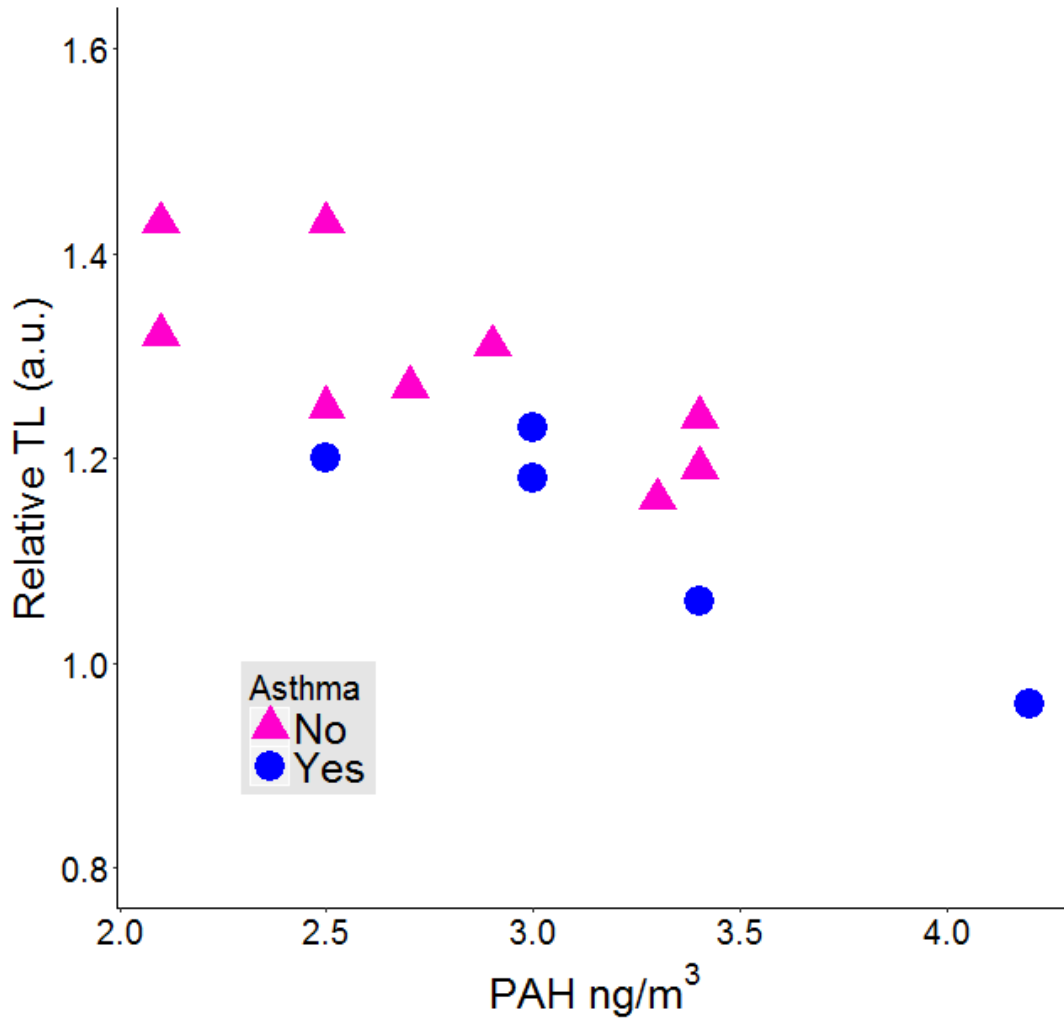


Figure 4. Scatter plot displaying the relative telomere length by asthma status (red triangle= non-asthmatics, blue circle= asthmatics) and PAH exposure.

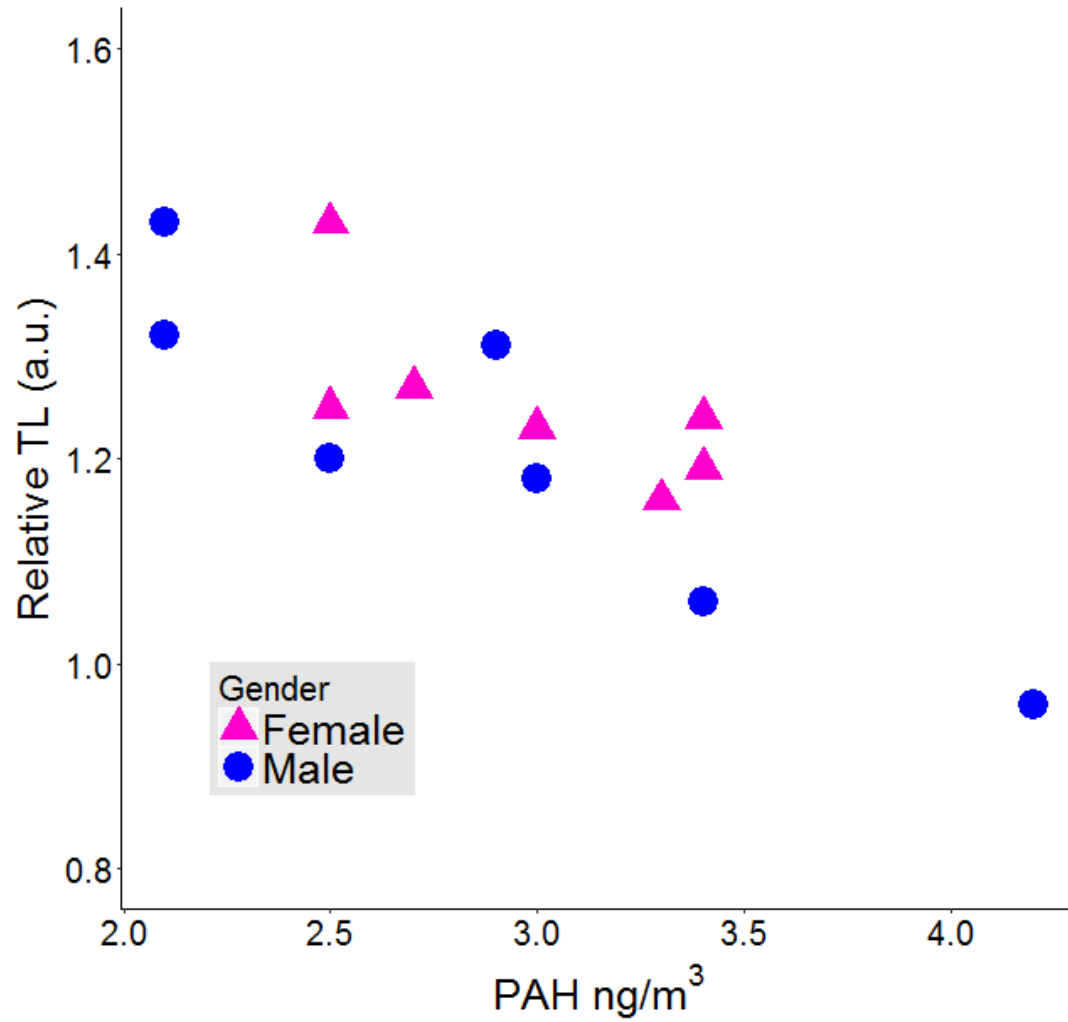


Figure 5. Scatter plot depicting the relationship between relative telomere length and PAH exposure by gender.

PREFACE FOR CHAPTER 3:

In the first study, we explored the association between ambient air pollution and asthma exacerbations. However, it was challenging to interpret the results without understanding the exact biological mechanism. Hence, we explored telomere length as a biomarker of oxidative stress caused by air pollution in a small pilot study (Chapter 2) and found evidence that exposure to ambient air pollution damages telomeres. Due to the small sample size, we were not able to make a conclusive statement about the observed results. In this chapter, we investigated the association between exposure to multiple air pollutants and telomere length with a larger sample size and adjusted for confounding factors. Based on the results from the second chapter, we hypothesized that exposure to ambient air pollution adversely affects telomere length in children. We found that exposure to ambient SO₂ was significantly associated with telomere length and the effect was greater among the participants older than the median age (14 yrs). The information from this study can be used to better understand the biological pathways from exposure to health outcome.

CHAPTER 3

Exposure to Ambient Air Pollution and Telomere Length in African American Children and Adolescents Living in the San Francisco Bay Area, United States. SAGE II study.

ABSTRACT

Rationale: The effect of air pollution on children's health may differ from that of adults due to their immature respiratory and immune systems. Understanding the biological mechanisms linking air pollution to disease outcomes in children is crucial to prevent and treat chronic diseases.

Objectives: In this study, we investigated the association between ambient air pollutants and telomere length in minority children in an attempt to understand the potential damage caused by air pollution at the molecular level.

Methods: Study participants came from an existing case-control study of African American children and adolescents with asthma in the San Francisco Bay Area (N= 1269). The annual average daily exposure to PM_{2.5}, NO₂, O₃ and SO₂ were used to investigate the association with telomere length after adjusting for confounding. We performed multiple imputation to replace missing covariate values. The relative telomere length for each participant was measured by uniplex polymerase chain reaction (PCR).

Results: Increasing SO₂ was associated with a significant decrease in telomere length ($\beta = -0.18$, 95% CI= (-0.02, -0.32)), whereas results for PM_{2.5}, NO₂, O₃ were null. The effect of SO₂ was almost three times greater ($\beta = -0.25$, 95% CI= (-0.03, -.48)) among the participants older than 14 yrs (median age). African ancestry was significantly associated with longer telomere length in younger participants ($\beta = 0.004$, 95% CI= (0.0002, 0.008)).

Conclusions: Exposure to SO₂ was significantly associated with shorter telomeres at low concentrations while traffic-related pollutants were not. Older children appeared to respond more to ambient SO₂ than younger children. SO₂ may be a surrogate for toxic chemicals emitted from industrial sources in the area. The inverse association with African ancestry may reflect a complex relationship between biological and social factors, age and telomere length.

3.1. BACKGROUND

Exposure to ambient air pollution can cause adverse health effects because of its harmful chemical contents. There is overwhelming evidence that exposure to air pollution is associated with the incidence and exacerbation of multiple diseases.¹⁻³ Based on animal models and toxicology studies, oxidative stress is a plausible biological mechanism to explain air pollution induced disease outcomes.^{4,5} However, the underlying pathways from exposure to adverse health outcomes are not fully understood.

Recent clinical and epidemiological studies suggest that telomere length can be considered as a new biomarker of oxidative stress and inflammation.⁶⁻⁸ Telomeres are chromosomal units composed of multiple copies of hexanucleotide (TTAGGG) at the ends of eukaryotic chromosomes. Maintenance of telomere length is essential for cell replication and viability because cells with short telomeres can no longer divide. This can interfere with regenerating essential cells in the human body. In addition, the high guanine contents of telomeres make them extremely vulnerable to oxidative stress. Therefore, telomere length can be used to reflect cellular history of oxidative stress and inflammation.

A number of clinical and in-vitro studies have presented high correlations between oxidative stress biomarkers and telomere shortening, which were then associated with chromosome instability and DNA damage as downstream effects.⁹⁻¹¹ In contrast, reducing telomere damage resulted in less chromosome instability.^{12,13} Exposure to traffic-related air pollution (i.e., particulate matter, elemental carbon, and polycyclic aromatic hydrocarbon (PAHs)) and hazardous waste exposure have been associated with short telomere length in adult populations.¹⁴⁻¹⁶ Early-exposure to traffic-related air pollutants during pregnancy has also been associated with telomere shortening in newborns.¹⁷ Therefore, we hypothesized that exposure to ambient air pollutants generates reactive oxygen species, which in turn accelerate telomere shortening in children and adolescents.

Understanding of telomere biology in children and adolescents is limited. Children may be more susceptible to the effects of air pollution than adults due to their immature development. Moreover, it is not clear how early exposure to air pollution may contribute to telomere damage in children and how this may impact their health later in the life course. In a previous small pilot study, we found that exposure to ambient PAHs was correlated with telomere length in children.¹⁸ Here, we carry out a more detailed study with a larger number of subjects and explore relationships with other risk factors. The main objective of this study was to investigate the association between long-term exposure to specific ambient air pollutants and telomere length in a cohort of African American children and adolescents. We first examined the relationship between air pollutants and telomere length and further probed effect modification by African ancestry and age for mechanistic insights. To the best of our knowledge, this is the first large study to explore the relationship between ambient air pollution and telomere length in minority children in the United States.

Our initial hypothesis was that exposures to all ambient air pollutants are associated with shorter telomere length. We examine several traffic-related pollutants (PM_{2.5}, NO₂, PAH) as well as SO₂, an indicator for toxic chemicals emitted from local industrial activities.

3.2. METHODS

3.2.1. Study population

The Study of African Americans, Asthma, Genes & Environments (SAGE II) is a case control study of African American asthmatic youth in the San Francisco Bay Area. It was initiated in 2008 to examine the complex genetic and socio-environmental contributors to asthma prevalence, control, and severity among minority children and adolescents. Participants are aged 8–21 years and were recruited through a combination of community and clinic-based recruitment at urban-based health maintenance organizations or community health centers. The parents and all four grandparents of the participants must self-identify as African Americans to be eligible for the study. Asthma is defined by physician diagnosis, report of symptoms, and asthma controller or rescue medication use within the last 2 years. Participants are excluded if they reported any of the following: 1) 10 or more pack-years of smoking; 2) any smoking within 1 year of recruitment date; 3) history of lung diseases other than asthma (cases) or chronic illness (cases and controls); or 4) pregnancy in the third trimester. All local institutional review boards approved the study and all parents/participants provided appropriate written consent/assent.

SAGE II has enrolled 1,556 participants (920 cases and 636 controls) from 2008 to August 2013. In this, we only included subjects with complete air pollution and telomeres data (n= 1269). We excluded participants with missing demographic or covariate information. The final analytical sample size was 719. Though collected for a case-control study of asthma, the data were analyzed as a cross-sectional study of telomere length and average daily exposure to air pollutants.

3.2.2. Estimation of ambient air pollution exposure

Each participant's exposures to ambient levels of SO₂, O₃, NO₂, and PM_{2.5} were estimated based on geocoded residential addresses and air quality data from regional monitoring stations. Each address in a participant's residential history was geocoded using TomTom/Tele Atlas EZ-Locate software (TomTom, Amsterdam, the Netherlands). Ambient air pollution data were acquired from the U.S. Environmental Protection Agency Air Quality System. From the recruitment date, we averaged the daily concentrations over the previous 12-month period to estimate annual average exposures by calculating the inverse distance-squared weighted average from the four closest air pollution monitoring stations within 50 km of the geocoded residence. The exposures of those who moved during the course of the year were weighted based on the number of months spent at each residence.

3.2.3. Telomere length measurement

Total genomic DNA was purified from peripheral blood mononuclear cells (PBMCs) using QIAamp® DNA Mini kit (QIAGEN, Cat#51104). The telomere length assay was adapted from the published original method by Cawthon.¹⁹ Relative telomere length was measured by using uniplex quantitative polymerase chain reaction (qPCR) in triplicate with the 36B4 gene as a reference/housekeeping gene. Samples with standard deviation greater than 1 cycle threshold (Ct) values (5%) were removed from further analysis. Relative telomere lengths were determined

by subtracting the mean single copy gene cycle threshold (Ct) values from the mean telomere gene cycle threshold values (Ct).

3.2.4. Statistical analysis

As a part of our model selection procedure, we fitted generalized additive models with penalized splines to allow for nonlinearity in the exposure-response relationships. Visual plots of the smoothed curves present the telomere length on the y-axis and exposure to air pollutants on the x-axis. All models were adjusted for confounding.

In the main analysis, air pollutant exposures and telomere length were treated as continuous variables. The association between annual average daily ambient air pollutant exposures and telomere length were assessed by multivariable linear regression models.

Baseline characteristics including age, sex, body mass index (BMI) and asthma status at the time of recruitment were treated as potential confounders and included in all models. Additional potential confounding variables were added if they were associated with both exposure and outcome variables and not on the causal pathway, including exposure to the other pollutants, recruitment season (spring, summer, fall, winter), composite socioeconomic status (SES), African genetic ancestry and cigarette smoking. A composite SES score was created based on maternal education, 12-month household income, and insurance level.³ We categorized the composite SES score into three categories (low, mid and high) based on tertiles of all subjects. For smoking, the number of smokers living in the home where child lives was used to create an ordinal variable (0= none, 1= one person, 2= two or more).

The final multivariable model adjusted for age, sex, BMI, SES score, recruitment season, African ancestry, asthma status and smoking. Then, we stratified the data into two age groups (below and above the median age, 14) to investigate effect modification by age.

We performed multiple imputation for missing data on maternal education, annual household income, insurance status, BMI, African ancestry and smoking. We first diagnosed the missing data patterns and used an algorithm to estimate and replace missing values for each variable. The algorithm in “mi” package was based on a chained equation approach (e.g. Bayesian framework).²⁰ For each missing value, the algorithm iteratively draws imputed values conditioned on the observed and imputed values of the other variables in the data.

The variables included in the data imputation process were telomere length, SO₂, NO₂, O₃, PM_{2.5}, age, sex, BMI, asthma status, recruitment season, ancestry, SES category, and smoking. “mi” R-package was applied to perform multiple imputation.²¹

All data analyses were done in R language (R Studio Version 1.0.136).

3.3. RESULTS

3.3.1. Study population

Baseline characteristics of SAGE II participants are shown in Table 1. The annual average exposure concentrations for PM_{2.5}, NO₂, and SO₂ in the residential areas of SAGE II participants were lower than the National Ambient Air Quality Standards (NAAQS). The San Francisco Bay Area is in “marginal non-attainment” status for the 2008 8-hour O₃ NAAQS (75 ppb).

We first examined the missing data patterns of 1269 participants. Missing values were imputed and replaced for the self-reported maternal educational level (2.4%), annual household income (20.4%), insurance status history (1.6%), BMI (23.8%), African ancestry (6.7%), and smoking (1.5%).

3.3.2. Air pollution and telomere length

The regression coefficients of exposure variables and covariates are displayed in Table 2. The only pollutant that was significantly associated with telomere length was SO₂. A one unit increase in ambient SO₂ exposure (ppb) was associated with -0.18 change in telomere length. Asthmatics had significantly shorter telomeres than control subjects by -0.14 units. Increasing age was associated with shorter telomeres. Participants who were either overweight or obese had shorter telomere lengths than their reference group (normal BMI). In addition, participants with low or middle socio-economic status scores had shorter telomere length than high SES participants. In contrast, having a greater proportion of African ancestry was significantly associated with decreased telomere shortening. The number of smokers currently living in the house was not associated with telomere length.

3.3.3. Effect modification by age

Effect modification was assessed by stratifying the exposure-response analysis at the 50th percentile age cut point (14 yrs), shown in Table 3.

In younger participants, exposures to PM_{2.5} and SO₂ were negatively associated with telomere length, however, none of the effect estimates were significant. In older participants, exposures to O₃ and SO₂ were negatively associated with telomere length. The effect of SO₂ was almost three times greater in older participants. The coefficient of African ancestry was large and significant only among the younger participants. The age-related decline in telomere length was identical in the two age groups.

3.4. DISCUSSION

In this study we assessed the effects of long-term exposure to air pollution on telomere length among minority children and adolescents living in a relatively low pollution urban environment in the U.S. Currently, there are very few studies of telomere biology in children, adolescents and young adults (8 to 22 yrs). This study aimed to address this knowledge gap. We hypothesized that exposure to all air pollutants would have adverse effects on telomere length. Instead, we discovered that only exposure to SO₂ was associated with telomere shortening, whereas the other pollutants were not associated with this outcome. Furthermore, the effect of ambient SO₂ was almost three times greater in the older participants. We also found that having a higher

proportion of African genetic ancestry was protective for maintaining telomere length. The association with ancestry was stronger among the younger subjects. The information from our study sheds some light on the extent of oxidative damage to chromosomes caused by ambient air pollutants and helps to establish a potential biological pathway from exposure to disease outcomes.

3.4.1. Air pollution and telomere length

Exposure to ambient SO₂ was significantly associated with telomere length after adjusting for confounding variables. Although the average concentration of ambient SO₂ exposure was generally low among SAGE II participants (1.04±0.3) it is possible that SO₂ also acts as an indicator of other unmeasured toxic pollutants emitted from local point sources. Figure 3 shows local point sources and their emission rates of SO₂ in our study area. The major sources of SO₂ include airports, petroleum refineries, gas and oil plants, calcined pet coke plants, electric power plants, cement manufacturing factories, a chemical plant and a landfill.²² Although we did not have direct measurements, according to the Environmental Protection Agency's national emissions inventory data, these facilities emit Volatile Organic Compounds (VOCs), heavy metals (lead, mercury, chromium, arsenic), formaldehyde, ethyl benzene, acrolein, 1,3-butadiene, 1,4-dichlorobenzene, and tetrachloroethylene into the air along with SO₂. These chemicals are highly toxic and inhaling a small amount may cause significant oxidative stress.²² We believe that in our study, SO₂ may be a proxy for exposure to this class of chemicals. The telomere shortening we observed possibly resulted from average daily exposure to all of these chemicals in the past year.

While SO₂ was significantly associated with shorter telomere length in this study, traffic-related pollutants appeared to have no damaging effect on telomeres. Our results were somewhat surprising because several previous studies have consistently demonstrated that long-term exposure to air pollutants significantly shortened telomere length and reduced telomerase activity in adults.²³⁻²⁵ One possible explanation for the inconsistency is that the toxic chemicals emitted from the industrial sources for which SO₂ is acting as a surrogate could be more potent oxidizing agents for telomere damage than traffic-related air pollutants. Another explanation is that both the lack of association between traffic-related air pollutants and the association with SO₂ with telomere length in our study cohorts may be related to unmeasured confounding by socioeconomic factors. If individuals with a higher SES and healthier lifestyle tend to live farther away from industrial sources and closer to traffic related pollution sources in the Bay Area, then the association between traffic-related air pollutants and telomere length is likely confounded by SES.

3.4.2. Effect modification by age, African ancestry, and asthma

We hypothesized that the effect of exposure to air pollution on telomere length would be greater among younger children due to their immature development. We found that the effect of ambient exposure to SO₂ was almost 3 times greater among participants older than 14 years compared to younger participants. Similar to the findings above, the associations between telomere length and exposure to traffic-related air pollutants were non-significant for both young and old participants.

A longer duration of exposure combined with age related telomere repair in older participants may have contributed to a greater total decrease in telomere length. It is also possible that the association we found is confounded by other telomere shortening risk factors experienced by older participants to a greater degree including psychological stress, poverty, and diet, and exposure to other environmental pollutants. However, telomere shortening rates were almost identical for both young and old participants. This suggests potentially differing telomere repair mechanisms between younger versus older participants because the same amount of SO₂ exposure had a greater impact on older participants.

One possible difference in repair mechanisms may be due to differing levels of telomerase activity in younger participants.^{26,27} It is likely that rapid physical development in younger children stimulates telomerase activity, which in part counterbalances the telomere loss. In younger children, damaged PBMCs can be replaced much faster because of the large reservoir of naïve cells in their thymus.^{29,29} The different telomere biology in younger children versus older children and adolescents may partially explain the greater impact of SO₂ in older participants.

We also found that African ancestry was positively associated with telomere length, which is consistent with other studies.³⁰⁻³³ The positive association of African ancestry was significant only among the younger participants. The association between African ancestry and telomere length is somewhat unexpected because African ancestry has been linked to increased expression of inflammation-related genes.³⁴ African ancestry is also associated with a higher prevalence of asthma in children which, in turn, was associated with shorter telomere length.³⁵⁻³⁷ Previous studies have consistently reported that individuals with African ancestry had longer telomere length than whites.³⁰⁻³³ It is likely that fewer replications of hematopoietic stem cells and progenitor cells in African Americans might have contributed to longer telomere length.³⁰ Further research is needed to understand the relationships between African ancestry, exposure to air pollution and telomere length.

The effect modification by age suggests that the loss of telomere length increases with continued exposure over time. This may be due to age-related telomere maintenance mechanisms and/or increased exposure to risk factors for telomere shortening as one gets older. Effect modification by African ancestry in children may reflect complex relationships between ancestry and inflammation related genes and environmental factors. These results open up possibilities for research into how age, African ancestry, and socio-economic factors tie into telomere shortening, and ultimately health outcomes.

3.4.3. Strengths and limitations

Ours is one of the very few studies that have investigated the relationship between environmental pollution and telomere length in children and adolescents living in the United States. We studied a large number of participants, which enabled us to explore how genetic variations and asthma status were associated with telomere length. Results from our study suggest that chemicals released from local point sources may possess a stronger oxidizing potential than pollutants from traffic sources.

One of the major limitations of our study was that the ambient air pollution exposure assessment was solely based on the residence of the subjects. The exposures of individual subjects may be either under- or over-estimated since we did not incorporate time-activity patterns into the model. However, any measurement error that occurred from the exposure assessment would be expected to be non-differential, and therefore, biased towards the null.

The cross-sectional design is another major limitation. Due to the nature of our study design, it is not possible to establish temporality, and thus, suggest a causal role of air pollutants in telomere shortening.

The unmeasured confounding of ambient exposure to SO₂ and telomere length by other stationary pollutants and SES is perhaps the most important limitation of this study. For example, African American boys who lived in a stressful home environment had shorter telomeres than children living in less stressful homes.³⁸ In a previous study that included SAGE participants, African American children who reported more perceived discrimination had increased risk of asthma and poorer control of asthma compared to African American children who did not report discrimination.³⁹ In future studies, it will be useful to consider psychosocial and neighborhood environmental factors as potentially important covariates.

3.5. CONCLUSIONS

We found that exposure to ambient SO₂ was associated with shorter telomere length while exposure to several traffic-related pollutants was not. We believe that SO₂ may be a surrogate for toxic chemicals emitted from local point sources in the area, which likely contribute to the observed association between SO₂ and telomere length. The lack of association between traffic-related pollutants and telomere length may arise from SES related factors. Participants with higher SES may be more likely to live away from industrial sources; therefore, their primary source of ambient air pollution would be road traffic emissions. Exposure to ambient SO₂ had greater impact in participants older than the median age. We also found that individuals with a higher percentage of African genetic ancestry were less susceptible to telomere damage. This protective effect was more significant in younger participants. Individuals with African ancestry may have longer telomeres due to fewer cell replications, which can be protective against oxidative stress and inflammation in early ages. However, this relationship can change with the presence of more stressors. The inverse effect of African ancestry may reflect complex inter-relationships between biological and social factors and age. Whether exposure to SO₂ may itself lead to shorter telomeres or is a marker of other exposures to toxic air contaminants emitted from point sources remains an important question with environmental justice implications, opening up many exciting avenues for further research.

TABLES

Characteristic (N=1269)	Mean	SD
Age (years) (8-22 yrs.)	14.4	3.7
BMI [†] (kg/m ²) (n=967)	24.6	7.0
Telomere length (delta delta)	4.6	0.7
African ancestry (%) (n=1183)	78.8	12.4
Annual daily air pollution exposures		
<i>PM_{2.5}</i> μg/m ³	9.3	1.9
<i>NO₂</i> ppb	12.3	3.2
<i>O₃</i> ppb	21.6	4.2
<i>SO₂</i> ppb	1.05	0.3
	N	%
Asthmatics	761	60.0
Gender (male)	625	49.2
BMI Category (n=967)		
<i>Underweight</i>	9	0.9
<i>Normal</i>	459	47.5
<i>Overweight</i>	199	20.5
<i>Obese</i>	300	31.0
SES Score (Ref: High) (n=979)		
<i>Low</i>	334	34.1
<i>Middle</i>	433	44.2
<i>High</i>	212	21.6
Smoking (n=1250)		
0= no smoker	864	69.1
1= one smoker	271	21.7
2 =two or more smokers	115	9.2

Table 1. Baseline characteristics of SAGE II participants (cases and controls).

*Values are shown as mean and SD for continuous variables and frequency and percentage for categorical variables.

[†]BMI was calculated as following, BMI = weight (kg) / (height (m))²

Variable Name (N=1269)	β	95% CI
Annual air pollution exposures		
<i>PM_{2.5}</i>	-0.0007	(-0.027, 0.026)
<i>NO₂</i>	0.01	(-0.006, 0.03)
<i>O₃</i>	0.001	(-0.01, 0.02)
<i>SO₂</i>	-0.18	(-0.02, -0.32)
Age	-0.01	(-0.02, 0.0006)
Gender (Ref: male)	-0.04	(-0.12, 0.03)
BMI (Ref: normal)		
<i>Underweight</i>	0.40	(-0.37, 1.10)
<i>Overweight</i>	-0.10	(-0.21, 0.02)
<i>Obese</i>	-0.07	(-0.16, 0.05)
SES Score (Ref: high)		
<i>Low</i>	-0.08	(-0.20, 0.06)
<i>Middle</i>	-0.08	(-0.19, 0.04)
Asthma status (Ref: control)	-0.15	(-0.06, -0.23)
African ancestry	0.004	(0.0002, 0.008)
Smoking	0.04	(-0.04, 0.15)

Table 2. Coefficients of regression model for telomere length, adjusting for age, gender, BMI, African ancestry, recruitment season, SES score, asthma status, number of smokers living at home, and exposure to PM_{2.5}, NO₂, O₃ and SO₂ , with imputed data.

Variable (N=1269)	<= 14 yrs (N=630)		> 14 yrs (N=639)	
	B	95% CI	β	95% CI
PM _{2.5}	-0.01	(-0.05, 0.02)	0.001	(-0.037, 0.04)
NO ₂	0.02	(-0.01, 0.05)	0.02	(-0.01, 0.05)
O ₃	0.004	(-0.02, 0.03)	-0.003	(-0.03, 0.02)
SO ₂	-0.08	(-0.28, 0.11)	-0.25	(-0.03, -0.48)
African ancestry	0.005	(0.0004, 0.01)	0.001	(-0.004, 0.006)
Age	-0.02	(-0.05, 0.009)	-0.02	(-0.06, 0.005)

Table 3. Effect modification by age (cut point of 14 years old) on the associations of exposure to ambient air pollutants, African ancestry, and age with telomere length after multiple imputation, adjusting for age, gender, BMI, African ancestry, recruitment season, SES score, asthma status, and number of smokers living at home.

FIGURES

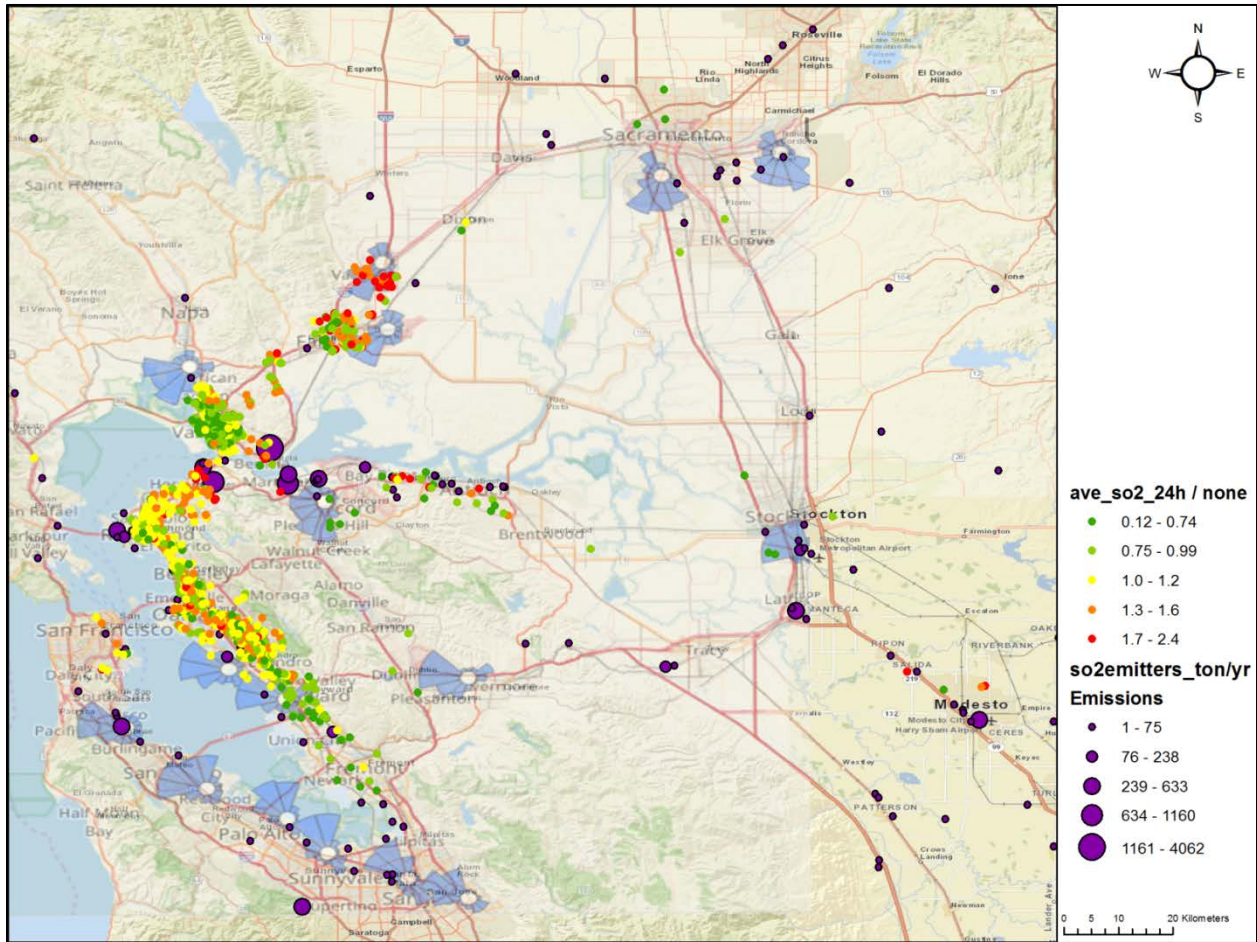
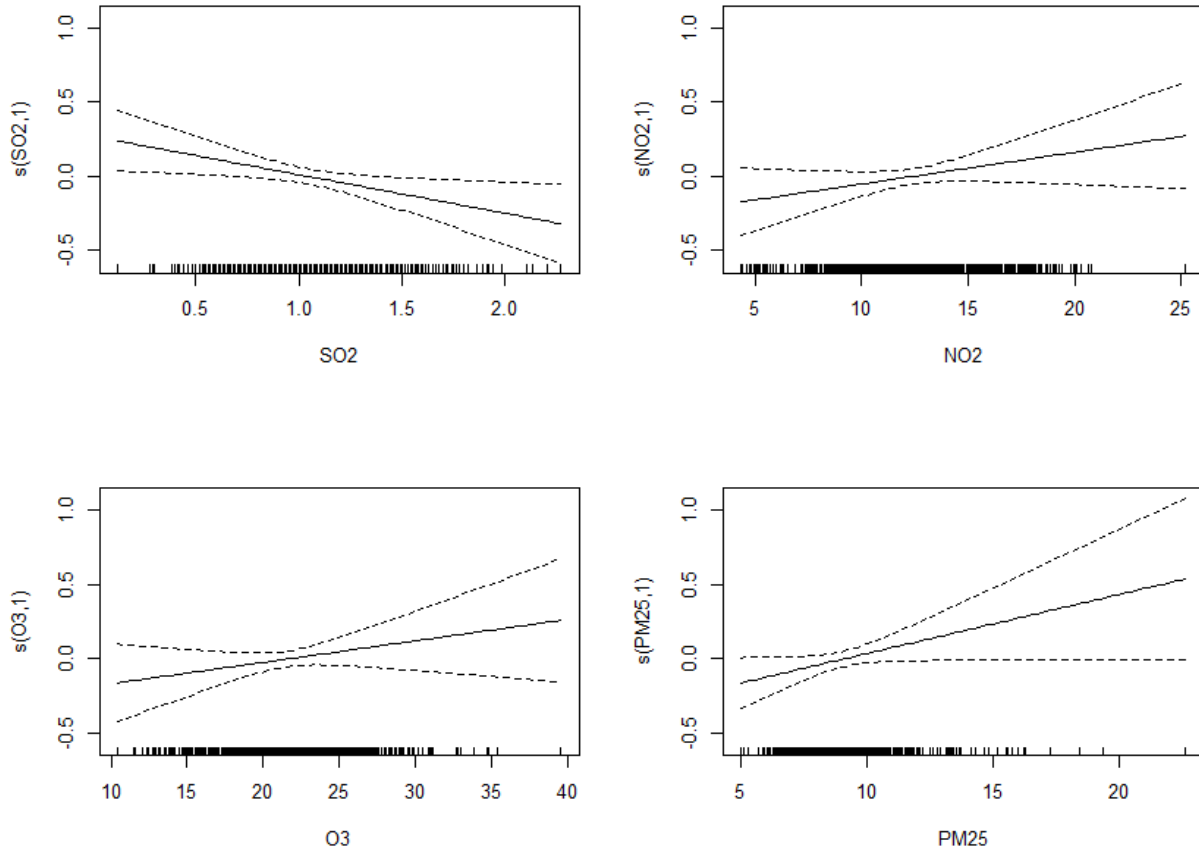
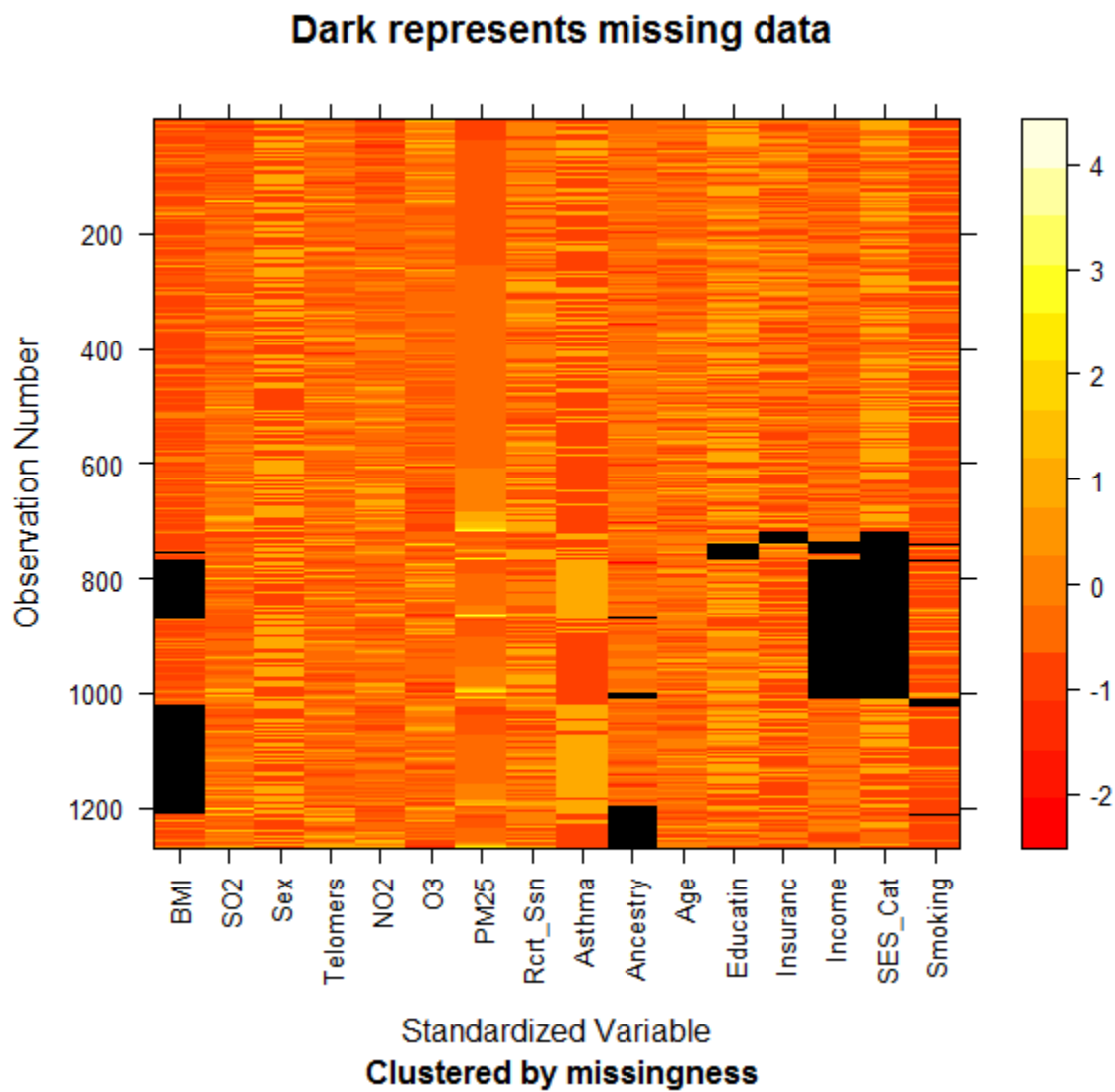


Figure 1. Prevalent wind directions are shown as blue wind roses. The annual average daily exposure to ambient SO₂ (ppb) for participants are shown in multiple colors (green as the lowest and red as the highest exposure levels). The intensity of SO₂ emission (ton/yr) from point sources is shown as dark purple circles.

SUPPLEMENTARY INFORMATION



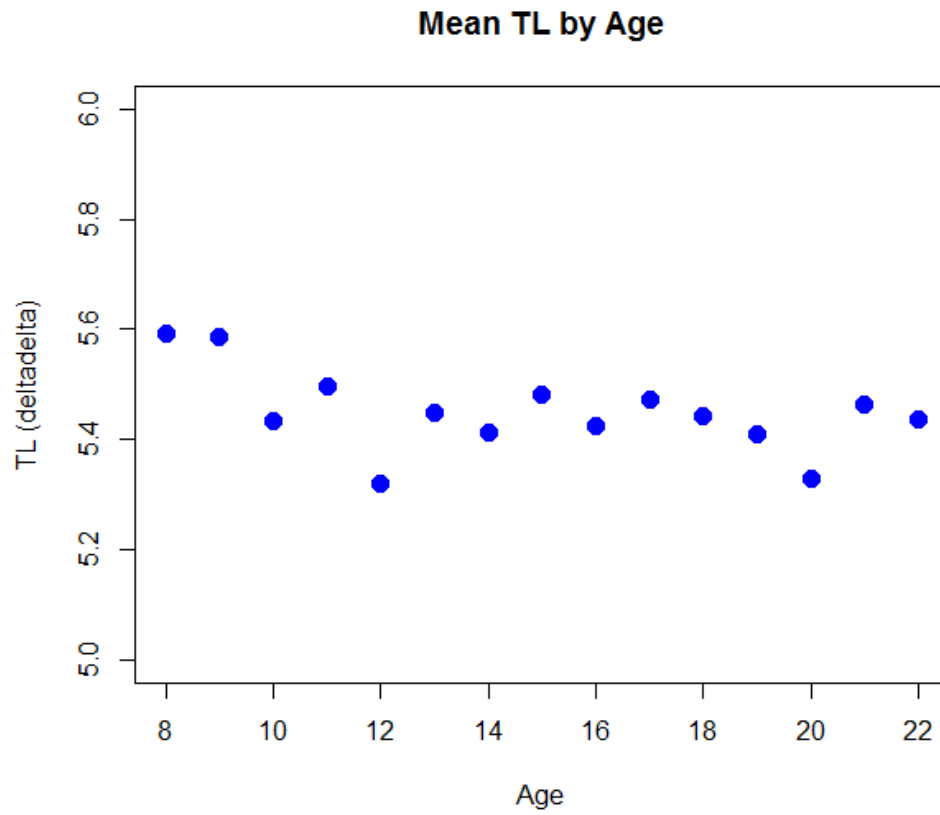
S.Figure 1. Generalized additive model for exposures to SO_2 , O_3 , NO_2 and $\text{PM}_{2.5}$ and telomere length. Models adjusted for age, BMI, sex, composite socioeconomic status (insurance level, 12-month average household income, maternal education level), asthma status, recruitment season, African ancestry, smoking and co-pollutants.



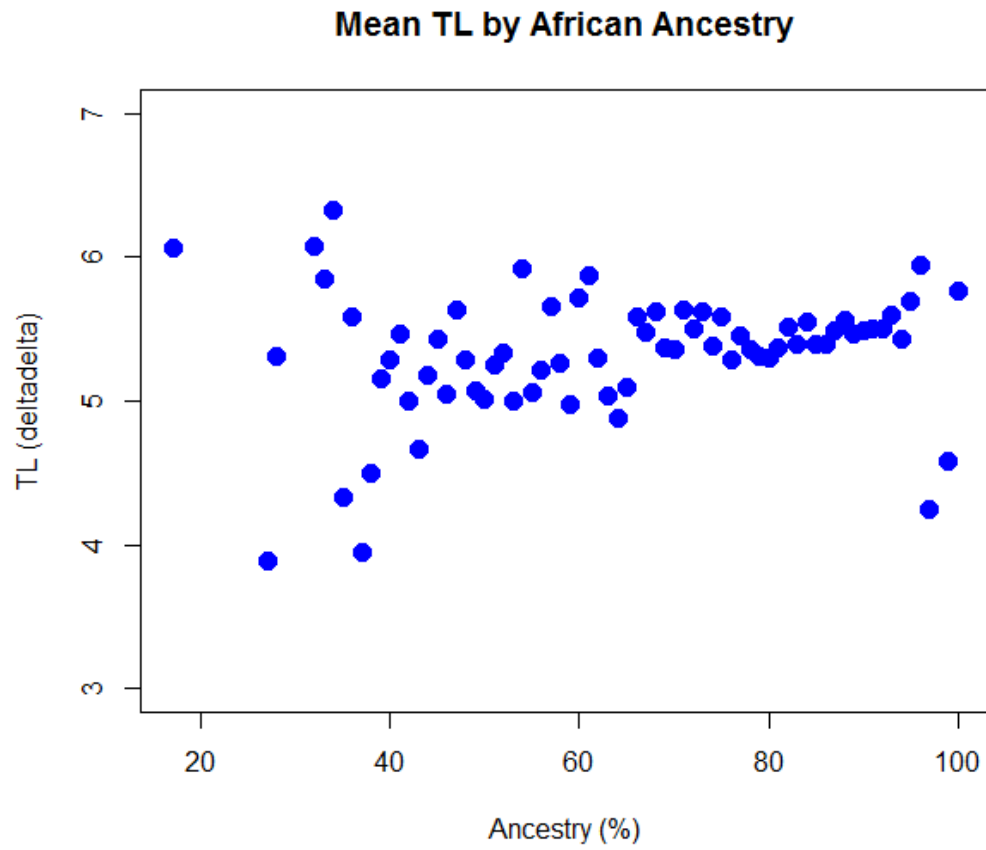
S.Figure 2. Missing data pattern shown in dark(black) color.



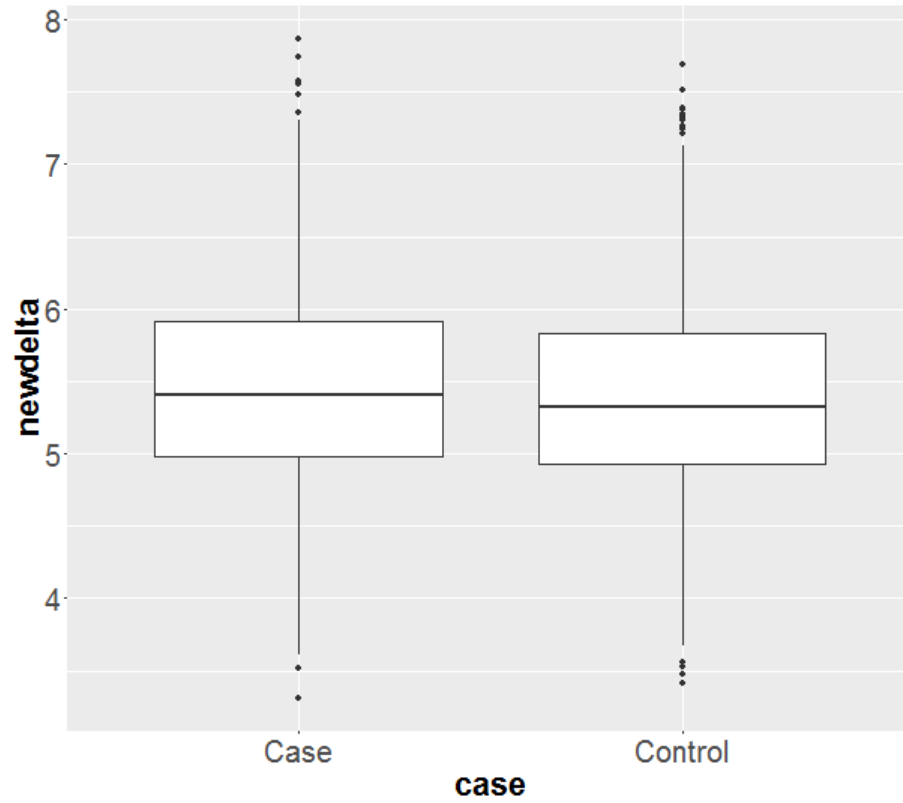
S.Figure 3. Correlation between air pollutants (SO₂, NO₂, O₃, PM_{2.5}).



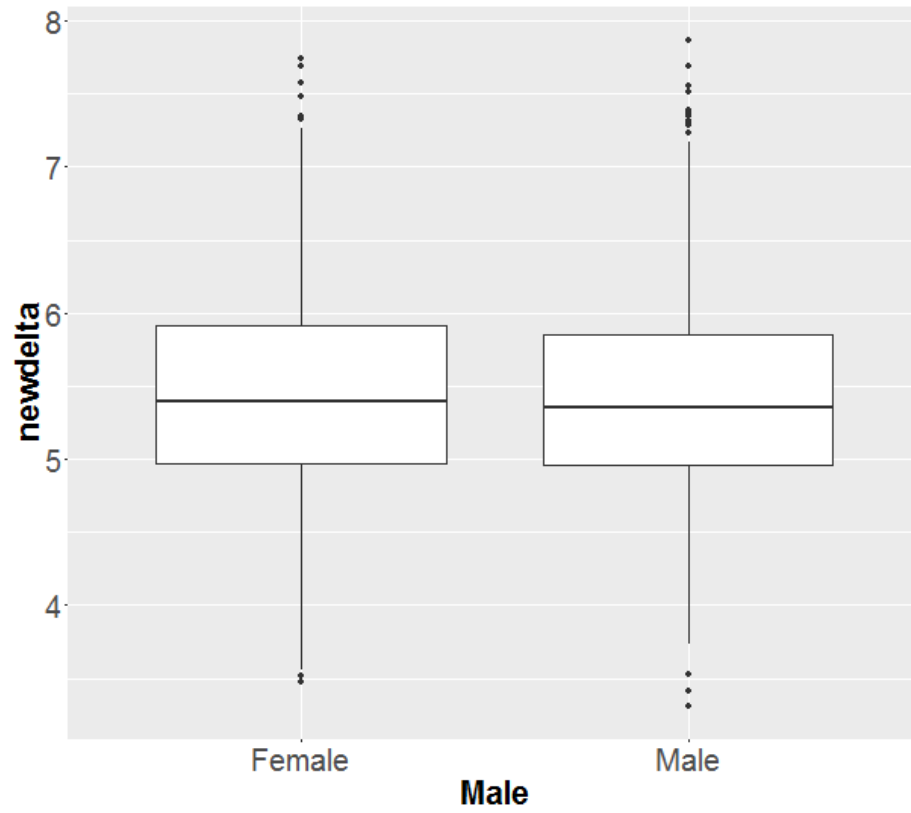
S.Figure 4. Scatter plot showing the relationship between age and relative telomere length.



S. Figure 5. Scatter plot showing the relationship between African ancestry and relative telomere length.



S.Figure 6. Box plots displaying 25th, 75th and median telomere lengths between asthmatic and non-asthmatic participants.



S.Figure 7. Box plots showing 25th, 75th and median telomere lengths by female and male participants.

CONCLUSIONS

This thesis had the overarching aims of 1) investigating whether the ambient air pollution is associated with negative health effects among urban minority children and 2) identifying potential biological pathways from exposure to health end points. Previous studies have demonstrated significant effects of air pollutants in both children and adults.¹⁻⁵ Animal models and clinical studies have been conducted to understand pathogenesis/pathophysiology of air pollution induced diseases; however, the exact underlying mechanisms are not yet clear.^{6,7} In this dissertation, I examined different mechanisms associated with various biological markers including asthma phenotype/endotype, genetic ancestry and telomere length.

The first chapter focused on the asthma exacerbation and potential exposure-response mechanisms that may be linked to the atopy status and genetic ancestry among four racial/ethnic populations of minority children and adolescents. We found that O₃ and NO₂ exposures were positively associated with asthma exacerbations after adjusting for potential confounding factors. However, results of PM_{2.5} were null in the cohort as a whole. In addition, we found differing inflammation mechanisms caused by O₃ exposure by atopy status. Lastly, our results indicated that participants with higher African ancestry may have more frequent asthma exacerbations. These findings suggest that air pollution is associated with asthma outcomes. Nevertheless, it was challenging to interpret the results without further explanation of biological mechanisms. Hence, we conducted additional studies to explore the potential pathways from exposure to health outcome by using a telomere length as a biomarker of oxidative stress. A number of studies have proposed that telomere length may be a biomarker of oxidative stress.¹¹⁻¹⁴ Moreover, telomeres' unique ability to control cell viability and chromosome stability can potentially be used to delineate biological mechanisms.

In the second chapter, we found that telomere length decreased with increasing PAH exposure among the small group of participants in a pilot study, consistent with the hypothesis that PAH exposure may cause oxidative stress that can accelerate telomere shortening. Our results also suggested that age, sex, and asthma status may influence the length of telomeres in children. Based on these results, we conducted a formal study with a larger number of subjects. We found that exposure to SO₂ was significantly associated with shorter telomeres at low concentrations while traffic-related pollutants were not. We speculate that SO₂ may be an indicator for toxic chemicals emitted from local point sources. In addition, older children appeared to respond more to ambient SO₂ than younger children. Lastly, it appeared that having a high percentage of African ancestry was associated with longer telomere length, which may reflect complex inter-relationships between biological and social factors, age and telomere length.

The findings in the first chapter, which looked at the association between air pollution and asthma exacerbation, build on previous research.^{1,2} A number of studies have shown that exposure to ambient air pollution was associated with asthma outcomes including worse lung function and increased use of asthma medication and hospitalization in both children and adults.³⁻⁵ Our results were consistent with these previous studies, except for our null results regarding effects of PM_{2.5}. The effect modification by atopy status suggests that it can be used to identify phenotype-specific pathobiological mechanisms useful for treating asthma patients.^{6,7} One of our group's early studies was not able to show strong evidence of interaction between

global genetic ancestry and air pollution.⁸ In our study, we have shown effect modification by African ancestry, which might be linked to the genetic variations within the oxidative stress pathways.^{9,10} The null results between traffic-related air pollutants and telomere length in my third chapter appear to be inconsistent with the findings in earlier studies that exposure to these air pollutants was significantly associated with shorter telomere length in adults.¹¹⁻¹³

It is recommended that our results should be interpreted with caution. First, due to the nature of our cross-sectional study design, the reported associations do not establish causality. We measured our outcome once and used the annual average daily air quality as the main exposure metric. Without establishing temporality, it is difficult to understand the causal role of air pollution. Second, our PM_{2.5} results in the first chapter were either null or conflicting with other results, which made it difficult to achieve the overall conclusion. The inconsistency between our results could have resulted from exposure misclassification since our exposure assessment was based solely on the participant's residence. Lastly, the residual confounding by SES and racial related factors has not been adequately identified and adjusted for in the analysis. Therefore, we cannot rule out that the observed associations were due to confounding.

My study offers suggestive evidence for negative effects of some specific air pollutants on asthma outcomes. In addition, the effect modification by atopy status indicates that individuals with non-atopic asthma may be more susceptible to ambient O₃ due to their underlying inflammatory mechanism. The interaction between ozone and African ancestry likely reflects a complex relationship between genetic and social factors. The implications of the second and third chapters support the argument for a different telomere repair/maintenance mechanism in younger children. Overall, our findings highlight the importance of identifying the biological pathways by which different air pollutants may impact asthma. Moreover, our telomere studies provide valuable information to bridge some of the linkages from exposure to health endpoints. Further studies are required to improve our understanding of those at particular risk and reduce the incidence and severity of air pollution-related disease outcomes.

For future studies, it is recommended to improve the exposure assessment by incorporating time activity-patterns or measuring air quality by personal monitors. Individual level exposure data can be used to understand spatial and temporal variability, and therefore, reduce exposure misclassification. In order to understand the biological mechanisms behind air pollution induced diseases, studies with more complex approaches are required. For example, admixture mapping can be used to find the genetic loci which may moderate the effects of ancestry on air pollution or the inflammatory markers to delineate mechanisms associated with an asthma phenotype. In addition, a longitudinal study design can improve our understanding about critical exposure windows during the developmental period and the temporal effects of telomeres on health outcomes.

The findings of this study add further evidence that exposure to ambient air pollution is likely a serious environmental risk factor that causes adverse health outcomes. In addition, my thesis investigated potential biological pathways that may lead to health outcomes from exposure to air pollutants. The effect modification by atopy status and genetic ancestry added to our knowledge in asthma phenotype associated inflammatory mechanisms and complex gene and environment interactions, which can impact asthma outcomes. Furthermore, my second and third chapters

were among the few studies investigating the association between air pollution and telomere length in children. These two studies gathered more information about how telomere length varied in relation to air pollution, gender, age, and asthma status. The study therefore advanced our knowledge of children's telomeres. Moreover, our results suggested that telomere length may be a new biomarker of oxidative stress induced by air pollution.

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