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Ambient Air Pollution, Asthma Drug Response and Telomere Length in African American Youth

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TITLE

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AUTHOR CONTRIBUTIONS

E.Y.L. conceived this work, performed data analyses and prepared the manuscript. E.A.E., J.R.B., E.G.B., and S.S.O. supervised this work. F.W.L. calculated all air pollution measurements and contributed to the interpretation of these measurements. E.A.E. contributed to data analysis. C.S.E. performed the telomere bioassay. S.L.S., E.N.B-B., A.D., K.E.M., M.A.L., L.N.B. and E.G.B. planned and supervised the collection of data from the various recruitment regions in the initial cohort. M.J.W., J.R.E., T.J.N., A.M.Z., K.L.K., A.C.Y.M., D.H., S.H., M.G.C., L-A.S. and P.C.G. contributed to interpretation of results, and provided revisions of the final manuscript.

The authors declare that they have no relevant conflicts of interest.

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ABSTRACT

Background

Telomere length can serve as a potential biomarker for conditions associated with chronic oxidative stress and inflammation such as asthma. Air pollution can induce oxidative stress. Understanding the relationship between telomere length, asthma, and air pollution is important for identifying risk factors contributing to unhealthy aging in children.

Objectives

To investigate the associations between exposures to ambient air pollutants and telomere length (TL) in African American children and adolescents, and to examine whether African ancestry, asthma status and steroid medication use alter the association.

Methods

Linear regression was used to examine associations between absolute TL and the estimated annual average residential ozone (O₃) and fine particulate matter (PM_{2.5}) exposures in a cross-sectional analysis of 1,072 children in an existing asthma case-control study. African ancestry, asthma status and the use of steroid medications were examined as effect modifiers.

Results

Participants' absolute TL was measured by quantitative polymerase chain reaction. A 1-ppb and a 1- $\mu\text{g}/\text{m}^3$ increase in annual average exposure to O₃ and PM_{2.5} were associated with a decrease in absolute TL of 37.1 kb (95% CI: -66.7 kb to -7.4 kb) and 57.1 kb (95% CI: -118.1 kb to 3.9 kb), respectively. African ancestry and asthma were not effect modifiers; however, exposure to steroid medications modified the relationships between TL and the pollutants. Past year exposure to O₃ and PM_{2.5} were associated with shorter TL in patients without steroid use.

Conclusion

Exposure to air pollution was associated with shorter telomere length in non-asthmatic children and adolescents. This was not the case for asthmatic children as a group, but those on steroid medication had less shortening than those not using steroids. Reduced exposure to air pollution in childhood may help to preserve telomere length.

CLINICAL IMPLICATIONS

Exposure to air pollution may be associated with shorter telomere length in non-asthmatic children. Among asthmatic children, steroid medication use appeared to reduce the risk of telomere shortening associated with exposure to air pollution.

CAPSULE SUMMARY

The results of this cross-sectional study suggest that exposure to O₃ and PM_{2.5} are risk factors for telomere shortening in non-asthmatic children. The use of inhaled corticosteroids may attenuate the risk of telomere shortening in children with asthma.

KEYWORDS

O₃; PM_{2.5}; telomeres; asthma; minority; children

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110

111 **ABBREVIATIONS**

112 TL: Telomere length

113 O₃: Ozone114 PM_{2.5}: Fine particulate matter with a diameter $\leq 2.5 \mu\text{m}$

115 DNA: Deoxyribonucleic acid

116 ROS: Reactive oxygen species

117 SAGE: The Study of African Americans, Asthma, Genes & Environments

118 qPCR: Quantitative real-time polymerase chain reaction

119 CI: Confidence interval

120 ppb: Parts per billion

121 kb: Kilo-base pairs

122 $\mu\text{g}/\text{m}^3$: Micrograms per cubic meter

INTRODUCTION

Telomeres are DNA-protein units composed of multiple copies of tandem nucleotide repeats (TTAGGG) that prevent chromosome fusion and DNA degradation by capping the ends of eukaryotic chromosomes.¹ With each cellular replication cycle, DNA at the end of the chromosome remains unduplicated, leaving a single-stranded overhang.^{1,2} Telomeres take the burden of this end-replication problem and shorten with each round of cell division. Short telomere length (TL) has been linked to increased risk for age-related diseases such as cancer, cardiovascular disease and chronic obstructive pulmonary disease.³⁻⁷ In addition to cellular replication, the rate of telomere shortening can be accelerated by oxidative stress and inflammatory mechanisms, such as those resulting from smoking, obesity, psychosocial stress, and certain diets.⁸⁻¹⁰

Exposure to ambient air pollution is a possible risk factor for telomere shortening. Inhaled pollutants produce reactive oxygen species (ROS) that cause oxidative stress in the airways.¹¹ This process could substantially impact TL because the high guanine content in telomeres confers sensitivity to ROS.^{11,12} Additionally, asthma is associated with increased oxidative stress in the airways, which exposure to air pollution can aggravate, thereby contributing to adverse asthma outcomes.¹³ Because TL has been proposed as a potential biomarker for persistent asthma, it is necessary to elucidate the relationships between asthma, air pollution, and TL.¹⁴

Recent studies in newborn and adult populations suggest that short TL is associated with exposure to traffic-related air pollutants.¹⁵⁻¹⁸ However, the effects of air pollution on telomere biology remain largely unstudied, particularly among minority children and adolescents. Most studies of TL have been done in adult populations⁵⁻¹⁰ and information pertaining to minority children remains limited. Currently, there are two published studies on exposure to outdoor air

pollution and TL in children and their findings are inconsistent.^{19,20} Yet previous studies reported that African Americans are more likely to reside in places closer to sources of air pollution,²¹⁻²⁶ which would make them more vulnerable to telomere damage. More research is required to understand the impact of air pollution exposure on TL during childhood. Telomere damage incurred while *in utero* and/or in childhood may persist into adulthood, which could affect the biological aging process and increase the risk for multiple disease outcomes later in life.

Many chronic diseases have been associated with people with African ancestry or race.^{27,28} In clinical practice and biomedical research, populations are often divided categorically into distinct racial/ethnic groups. In reality, these categories are based on social rather than biological constructs and, comprise diverse groups with highly heterogeneous histories, cultures, traditions, religions, social and environmental exposures and shared ancestral background. While the factors captured by these categories contribute to clinical practice and biomedical research, the use of race/ethnicity is widely debated. Telomere length has been reported to vary by race/ethnicity.^{29,30} Therefore, we explored the relationship between quantitatively measured genetic ancestry and TL in our study population of young African Americans in addition to self-identified race/ethnicity.

Herein, we used case-control data to examine the association between TL and long-term average daily exposures to ozone (O₃) and particulate matter with a diameter ≤ 2.5 μm (PM_{2.5}) in a population of African American children and adolescents with and without asthma living in the San Francisco Bay Area. Our study was designed to address a knowledge gap in the existing air pollution and TL literature by providing information relevant specifically to minority children and adolescents. We hypothesized that exposure to ambient air pollutants is associated with shorter telomere length in African American children and adolescents. Furthermore, we

examined whether the association varies with African ancestry, asthma status and steroid medication use. This is the first large study to explore the relationship between ambient air pollution and TL in minority children and adolescents in the United States.

METHODS

Study population

This analysis examined a subset of subjects from the Study of African Americans, Asthma, Genes & Environments (SAGE)³¹⁻³³ with complete data on air pollution, telomere length and covariates specified below in the statistical analysis. After excluding 638 individuals who had missing data, a total of 1,072 subjects (aged 8-21 years), both asthma cases and controls, were used in this analysis (Table 1). Eligibility required all participants self-identify as African American. In addition, all four biologic grandparents were required to be identified as African American. Information about the SAGE study and subject selection criteria are outlined in Text E1 and descriptive statistics for subjects included and excluded in the analysis are summarized in Table E1.

All local institutional review boards of participating centers approved the study and all participants (or parents of participants younger than age 18 years) provided written informed consent.

Determination of absolute telomere length

Genomic DNA was isolated from whole blood according to manufacturer's recommendation using Wizard® Genomic DNA Purification Kits (Promega, Fitchburg, WI). Genomic DNA was assessed via agarose gel for DNA degradation; all samples demonstrated uniform intensity (>

10kb), and no smears were observed, indicating intact DNA samples. A NanoDrop® ND-1000 spectrophotometer (Thermo Scientific) was used to assess the quality and quantity of the extracted DNA. All samples assayed had absorbance ratios (260/280) between 1.8 and 2.0. Absolute TL was measured in triplicate by determining the number of TTAGGG hexamer repeats using quantitative real-time polymerase chain reaction (qPCR) as previously described (Text E2).³⁴ The use of the qPCR method is a widespread practice in air pollution, asthma and TL association studies due to its ability to generate high throughput TL data.^{35,36} More information about the absolute telomere length measurement can be found in the supplementary material.

Assessment of ambient air pollution exposure

We focused on O₃ and PM_{2.5} as the main air pollution exposures due to their ability to form free radicals and cause oxidative damage.¹¹ The concentration of ambient O₃ and PM_{2.5} were predicted by air pollution exposure models at the individual level. Each participant's exposure to ambient levels of O₃ (ppb) and PM_{2.5} (μg/m³) was assessed based on geocoded residential addresses at the point of enrollment and air quality data from regional monitoring stations as previously described (Text E3).^{28,37} We defined the annual average exposure as the average of daily air pollutant measurements over the 12-month period preceding recruitment.

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 designed to capture known genetic variation in specific populations.³⁸ Estimates of genome-wide African and European ancestries were obtained by means of a supervised analysis using ADMIXTURE assuming two ancestral populations.³⁹ Genotype data from European (CEU) and African (YRI) samples from

HapMap phase II were included as reference.⁴⁰ African ancestry was converted to represent percent (%) and considered as a potential confounder and effect modifier.

Statistical analysis

Characteristics for SAGE participants were summarized using the mean (standard deviation) for continuous variables and frequency (proportion) for categorical variables. Linear regression was used to model absolute TL as a function of air pollutant exposures, adjusting for age, sex, African ancestry, asthma status, number of household smokers, recruitment season, and maternal education. We also adjusted for telomere plate ID as a covariate to control for assay variation. A more detailed description on how the covariates were selected for inclusion in the final regression models can be found in Text E4. Consistent with previous studies, O₃ and PM_{2.5} exposure variables were both included in each model.^{28,37} The correlation between past year O₃ and PM_{2.5} exposures was -0.55.

African ancestry, asthma status and steroid medication use were also considered as potential effect modifiers. For each potential modifier, an interaction term was added to the regression model to test statistical significance: African ancestry (0= below median African ancestry; 1= above median African ancestry), asthma status (0=control; 1=case), inhaled corticosteroids (ICS) use (0=no; 1=yes), and any steroid use (ICS or/and oral steroids) in the past year (0=no; 1=yes). The regression coefficients of air pollution exposure were estimated in stratified analyses to further examine the magnitude of the differences in exposure-response between subgroups.

In our analysis, absolute TL was treated as a continuous variable and the range of absolute TL varied from 400 to 8,693 kbp. Since absolute TL was not normally distributed, we performed analyses on log-transformed absolute TL, which passed the Shapiro-Wilk test for normality.

Effect estimates were back-transformed and reported as change in mean absolute TL (kb/diploid).

All data analyses were done in the R statistical computing language (R Studio version 1.1.383).

RESULTS

Characteristics of the study population

The demographic characteristics of the 1,072 study participants are shown in Table 1. Mean age was 14.4 years (± 3.6) and ranged from 8 to 21 years. Average genetic African ancestry was 77.6% (± 12.3). There were more asthma cases (62.0%) than non-cases. Approximately, 59.4% of the study subjects' mother obtained a high level of education. In SAGE participants, mean absolute TL was 2,490.4 ($\pm 1,265.1$) kb. Subjects included in the analysis were more likely to be male, asthmatic and have a lower African ancestry score than those excluded due to incomplete data (Table E1). Average absolute TL was similar across recruitment seasons, though participants recruited in the fall had modestly longer telomeres (Table E2). Pollutants were slightly higher in the summer (Table E2).

Associations between long-term exposures to ambient air pollutants and absolute TL

The associations with annual average daily exposures to air pollutants in the full study population are presented as a change in absolute TL in kb per 1-ppb increase in O_3 and per 1- $\mu g/m^3$ increase in $PM_{2.5}$ (Table 2). After adjusting for covariates, absolute TL decreased by 37.1 kb (95% CI: -66.7 to -7.4) for each 1 ppb increase in annual average exposure to O_3 . The inverse association with each 1- $\mu g/m^3$ increase in annual average exposure to $PM_{2.5}$ of -57.1kb (95% CI: -118.1 to 3.9), was not statistically significant.

Asthma was not a significant effect modifier for air pollution exposure and TL (Table E3). The impact of past year exposure to O_3 and $PM_{2.5}$ on TL was significant only in participants without asthma (Table 3). Absolute TL decreased by 89.5 kb and 206.2 kb with every 1-ppb and $1-\mu g/m^3$ increase in O_3 and $PM_{2.5}$ exposure levels, respectively (95% CI_{O_3} : -147.7 kb to -31.2 kb; 95% $CI_{PM_{2.5}}$: -348.8 kb to -63.7 kb).

We observed that steroid medication use interacted with $PM_{2.5}$ exposures among asthma cases (Table 4). The additive associations of the interaction terms suggest that the use of ICS resulted in 164.1 kb (95% CI: 18.6 to 309.6) longer TL with a $1-\mu g/m^3$ increase in $PM_{2.5}$ exposure. The use of any steroid in the last 12 months was also associated with 175.9 kb (95% CI: -1.3 to 353.0) longer TL at marginally significant level ($p=0.06$) with a $1-\mu g/m^3$ increase in $PM_{2.5}$. The results for interactions between O_3 exposure and steroids use were null.

In stratified analyses (Table 5), the magnitude and the direction of the associations varied by the type of steroid medication use. While the results for ICS and any steroid use were not statistically significant, the general trends of the associations were towards longer TL. Among the non-ICS users, a 1-ppb and a $1-\mu g/m^3$ increase in past year exposure to O_3 and $PM_{2.5}$ were significantly associated with 86.0 kb and 190.9 kb decrease in TL, respectively (95% CI_{O_3} : -171.0 kb to -1.0 kb; 95% $CI_{PM_{2.5}}$: -374.3 kb to -7.4 kb). The impacts of air pollution exposure on TL were the greatest in magnitude among the asthma patients without any history of steroid use. The annual average exposure to O_3 and $PM_{2.5}$ decreased absolute TL by 123.5 kb and 314.9 kb, respectively, (95% CI_{O_3} : -248.4 to 1.4 kb; 95% $CI_{PM_{2.5}}$: -594.2 to -35.7 kb) with every 1-ppb and $1-\mu g/m^3$ increase in O_3 and $PM_{2.5}$ exposure levels, respectively.

We also considered adjusting for the duration of asthma (years), however, the inclusion of that term did not affect any of the associations between past year exposures to air pollutants and TL among the asthma cases (Table E4).

Association between African ancestry and absolute TL

After adjusting for covariates, a 1-percent increase in African genetic ancestry was associated with 8.4 kb longer absolute TL (95% CI: 0.8 to 15.9 kb) (Table 2). The associations between air pollution exposure and TL did not vary by different proportions of African ancestry (data not shown).

DISCUSSION

The results of our study suggest that exposure to ambient air pollution among African Americans during childhood and adolescence may increase the risk of telomere shortening. For example, we found that the annual average exposure to O₃ was associated with shorter absolute TL. Considering that one year of aging was associated with a 15.8 kb to 26.1 kb decreases in absolute TL (Table E5), a 1-ppb increase in O₃ exposure would be equivalent to 1.4 to 2.3 years of biological aging. Effect modification by asthma was not statistically significant. However, further analyses in asthma patients revealed that the use of inhaled corticosteroids, or any steroid, reduced the associations between air pollution and TL. We did not find any evidence for effect modification by African ancestry.

Annual average exposures to O₃ and PM_{2.5} were associated with decreased absolute TL, but the association with PM_{2.5} was not statistically significant. One possible explanation for this null finding is that ambient PM_{2.5} is a mixture of substances generated from chemical and biological

sources.^{41,42} Transition metals and organic molecules attached on the surface of fine particulate matter can induce the generation of free radicals in the airways, which can cause telomere shortening via an oxidative stress mechanism.^{11, 43,44} Certain bio-aerosols such as fungal spores and pollen have also been shown to produce reactive oxygen species.⁴⁵ Evidence from previous studies suggests that the composition and size of ambient particles, more so than their mass concentration, are associated with oxidative potential and harmful effects.^{46,47} Because the chemical, physical and biological composition of ambient particles in the San Francisco Bay Area may vary with time and location, we speculate that the annual average PM_{2.5} exposures we studied reflect different particle compositions depending on residential location and time of year. Another possible explanation for the lack of a significant association between past year exposure to PM_{2.5} and absolute TL is further explored below.

The associations between exposure to air pollution and absolute TL were significant only among the non-asthmatic participants and the magnitudes were stronger than among asthma cases. This finding was counterintuitive to our initial hypothesis. However, when we stratified asthma cases by the use of inhaled or any steroid use, the past year exposure to O₃ and PM_{2.5} resulted in significantly shorter TL among the non-ICS users. Moreover, the associations of O₃ and PM_{2.5} were the greatest in magnitude among the asthma patients who reported no steroid use over the last 12 months. The interaction results shown in Table 4 suggest that a 1- $\mu\text{g}/\text{m}^3$ increase in PM_{2.5} exposure is associated with a significant increase in the absolute TL of 164.1 kb in the asthma patients using ICS. The positive interactions between PM_{2.5} and steroids use can be interpreted in several ways: use of steroids leads to telomere elongation; use of steroids is protective against PM_{2.5} exposure; or some other factor associated with steroid use, such as severity of asthma, is playing a role. The exact mechanism of telomere elongation with steroid medication use is not

clear and beyond the scope of this manuscript. While not a study of the effect of corticosteroids, Townsley et al. have reported that androgen anabolic steroid therapy was effective in reducing telomere loss and lengthening telomeres, possibly mediated by up-regulation of telomerase activities, in patients with genetically defective telomeres.⁴⁸ One possible mechanism by which ICS could attenuate the effect of air pollution on TL in peripheral blood mononuclear cells is through decreased airway inflammation in response to pollutant-induced oxidative stress and less spillover of inflammatory and oxidative stress mediators into the systemic circulation. It is not clear why the use of steroid medication interacted with PM_{2.5} only. Other studies also report protective effects of steroid medication use against exposure to particulate matter, however, this was not the case for gaseous air pollutants.^{49,50}

The varying levels of African, European, and Native American ancestry in African Americans have important clinical and scientific implications for disease classification and gene discovery. One of our previous studies has demonstrated that the African American children were more likely to develop asthma than their Latino neighbors when they were exposed to equal levels of air pollution.²⁸ Two of our earlier works have found an inverse relationship between the percentage of African ancestry and lung function in African Americans and Latinos.^{51,52} Therefore, the next logical step was to investigate whether African ancestry modified the association between air pollution and TL. In this study, a 1-percent increase in African ancestry was associated with 8.4 kb longer TL. The presence of African ancestry as a continuous or a binary variable did not alter the association between exposure to air pollution and absolute TL. One possible explanation is that the negatively skewed distribution of African ancestry (the mean African ancestry = 77.6%) meant that there was limited variability within our study participants resulting in decreased statistical power to observe an association.

Age-dependent leukocyte TL shortening is due to successive divisions of hematopoietic stem cells (HSCs) and progenitor cells (PCs) that form peripheral leukocytes. On average, individuals of African descent have a lower number of circulating neutrophils than individuals of Caucasian descent. Longer TL in African American children than in white children is likely a result of fewer replications of HSCs/PCs.⁵³

We observed an inverse association between TL and age (Table 2). However, it may require a wider age range than our study to observe a significant age effect (Figure E1, Figure E2, and Table E6).

Strengths and limitations

Our study is one of the very few that has investigated the relationship between exposure to air pollution and absolute TL in children and adolescents living in the United States. We studied a relatively large population of well-characterized participants using established methods. Absolute TL has been reported to vary by self-identified race/ethnicity. Genetic ancestry covaries with self-identified race/ethnicity. Therefore, we quantitatively measured genetic ancestry, which enabled us to explore how large-scale ancestral genetic variation affects absolute TL. The average levels of the past year exposure to O₃ and PM_{2.5} in our study was below U.S. national ambient air quality standards. Our study results suggest that even relatively low-level air pollution may adversely affect absolute TL in children and adolescents. In addition, our study is the first to report a significant interaction between annual average exposure to air pollution and steroid use on absolute TL in minority children and adolescents with asthma.

A limitation of our study was that air pollution exposure assessment was based on study participants' residential addresses. Though our exposure assessment is widely used in large-scale

air pollution epidemiological studies,⁵⁴⁻⁵⁶ the exposures of individual subjects may be under- or overestimated since we did not incorporate time-activity patterns or indoor exposure into the model. However, any measurement error that occurred from exposure assessment would likely be non-differential, and therefore, lead to an underestimation of the true effect size. Integrating personal air quality monitor/sensor with air pollution exposure models would be the most ideal method to accurately measure the exposure levels to ambient and indoor air pollution. However, the application of personal air quality monitors to large-scale population studies like ours or even in a subset of study participants is not yet feasible. Additionally, there are privacy concerns and logistics that such monitoring entails. For future studies, incorporating time-activity patterns or using personal monitors to measure air quality at least in a subset of participants may improve exposure assessment.

Because our study used a cross-sectional analysis we cannot make strong inference about the role of either air pollution on TL or any effect modification by steroid use. A prospective study design of the effect of ICS on the air pollution-TL relationship would improve our understanding.

CONCLUSIONS

This study is the first to investigate the association between exposure to ambient air pollution and absolute TL in African American children and adolescents living in an urban setting. We discovered that the use of inhaled or oral steroids for asthma therapy was an important effect modifier. Steroid therapy appeared to reduce the harmful effects of air pollution, especially ICS, long-term treatment, among children with asthma. We found that annual average exposure to O_3 and $PM_{2.5}$ are important risk factors for biological aging in children and adolescents, which could potentially influence subsequent health outcomes. Reducing exposure to ambient air pollution

393 could improve telomere length maintenance, which may be associated with improved health
394 outcomes in African American children and adolescents.

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Table 1. Descriptive Statistics for SAGE Participants.

Characteristic	(N = 1,072)
Age, mean (SD), yrs	14.4 (3.6)
Male, N (%)	535 (50.0)
% African ancestry, mean (SD)	77.6 (12.3)
Asthma cases, N (%)	664 (62.0)
Current smokers in household, N (%)	
<i>None</i>	726 (67.7)
<i>One</i>	240 (22.4)
<i>Two or more</i>	106 (9.9)
Recruitment season, N (%)	
<i>Spring</i>	271 (25.3)
<i>Summer</i>	416 (38.8)
<i>Fall</i>	165 (15.4)
<i>Winter</i>	220 (20.5)
Maternal education, N (%)	
< <i>High school</i>	142 (13.2)
= <i>High school</i>	293 (27.3)
> <i>High school</i>	637 (59.4)
Telomere length, mean (SD), kb/diploid	2,490.4 (1,265.1)
Past year average, mean (SD)	
<i>O₃, ppb</i>	21.6 (4.1)
<i>PM_{2.5}, µg/m³</i>	9.3 (1.9)
Steroid medication in asthma cases, N (%)	
<i>Inhaled corticosteroids(ICS) use</i>	349 (52.6)
<i>Any steroid use</i>	446 (67.2)

O₃: ambient ozone. **PM_{2.5}**: particulate matter less than 2.5 µm in diameter. **ppb**: parts per billion. **µg/m³**: micrograms per cubic meter.

The proportions may not sum to 100% due to round-up error.

Table 2. Association Between Absolute Telomere Length and Annual Average Exposures to O₃ and PM_{2.5} 2006-2014.

Variable Name (n= 1,072)	Absolute Telomere Length [95% CI], kb	P-value
O ₃ , ppb	-37.1 [-66.7 to -7.4]*	0.01
PM _{2.5} , µg/m ³	-57.1 [-118.1 to 3.9]	0.07
African Ancestry, %	8.4 [0.8 to 15.9]*	0.03
Age, yrs	-26.1 [-52.3 to 0.1]	0.05

O₃: ambient ozone. **PM_{2.5}**: particulate matter less than 2.5 µm in diameter. **pbb**: parts per billion. **µg/m³**: micrograms per cubic meter.

Estimates provided as a change in mean absolute telomere length (kb/diploid) for 1-ppb and 1-µg/m³ increases in O₃ and PM_{2.5}, respectively, for annual average (past year) exposures. Multivariable linear regression model was adjusted for air pollutants (O₃ and PM_{2.5}), age, sex, African ancestry, asthma status, number of household smokers, recruitment season, and maternal education.

* p-value <0.05

Table 3. Association Between Absolute Telomere Length and Annual Average Exposures to O₃ and PM_{2.5} by Asthma Status: 2006-2014.

Exposure	Absolute Telomere Length [95% CI], kb	
	Asthma cases (N =664)	Non-asthmatics (N=408)
O ₃ , ppb	-17.4 [-53.6 to 18.9]	-89.5 [-147.7 to -31.2]*
PM _{2.5} , µg/m ³	-12.5 [-80.0 to 54.9]	-206.2 [-348.8 to -63.7]*

O₃: ambient ozone. PM_{2.5}: particulate matter less than 2.5 µm in diameter. pbb: parts per billion. µg/m³: micrograms per cubic meter.

Estimates provided as a change in mean absolute telomere length (kb/diploid) for 1-ppb and 1-µg/m³ increases in O₃ and PM_{2.5}, respectively, for annual average (past year) exposures. Multivariable linear regression models were adjusted for air pollutants (O₃ and PM_{2.5}), age, sex, African ancestry, number of household smokers, recruitment season, and maternal education.

*p-value <0.05

Table 4. Interaction between exposure to air pollution and steroid use on absolute TL among the asthma cases.

Interaction	N _{asthma cases} = 664	aTL [95% CI], kb	P-value
O ₃ × ICS [†]	n=349	-6.9 [-59.5; 45.7]	0.79
PM _{2.5} × ICS [†]	n=349	164.1 [18.6; 309.6]	0.03*
O ₃ × any steroid [¶]	n=446	3.9 [-54.1; 61.9]	0.89
PM _{2.5} × any steroid [¶]	n=446	175.9 [-1.3; 353.0]	0.06

O₃: ambient ozone. **PM_{2.5}**: particulate matter less than 2.5 μm in diameter. **ppb**: parts per billion. **μg/m³**: micrograms per cubic meter.

Estimates provided as a change in mean absolute telomere length (kb/diploid) for 1-ppb and 1-μg/m³ increases in O₃ and PM_{2.5}, respectively, for annual average (past year) exposures.

[†]Multivariable linear regression models were adjusted for air pollutants (O₃ and PM_{2.5}), age, sex, African ancestry, number of household smokers, recruitment season, maternal education, ICS use, and air pollutant × ICS use interaction term.

[¶]Multivariable linear regression models were adjusted for air pollutants (O₃ and PM_{2.5}), age, sex, African ancestry, number of household smokers, recruitment season, maternal education, any steroid use and air pollutant × any steroid use interaction term.

* p-value <0.05

Table 5. Association Between Absolute Telomere Length and Annual Average Exposures to O₃ and PM_{2.5} by Steroid Use Subgroups: 2006-2014.

Exposure	Absolute Telomere Length [95% CI], kb	
	ICS (n=349)	Non-ICS (n=315)
O ₃ , ppb	12.6 [-23.2 to 48.4]	-86.0 [-171.0 to -1.0]*
PM _{2.5} , µg/m ³	34.3 [-24.8 to 93.4]	-190.9 [-374.3 to -7.4]*
	Any steroid (n=445)	
	Non-steroid (n=219)	
O ₃ , ppb	1.95[-32.5 to 36.4]	-123.5 [-248.4 to 1.4]
PM _{2.5} , µg/m ³	26.2[-33.7 to 86.1]	-314.9 [-594.2 to -35.7]*

O₃: ambient ozone. **PM_{2.5}**: particulate matter less than 2.5 µm in diameter. **ppb**: parts per billion. **µg/m³**: micrograms per cubic meter.

Estimates provided as a change in mean absolute telomere length (kb/diploid) for 1-ppb and 1-µg/m³ increases in O₃ and PM_{2.5}, respectively, for annual average (past year) exposures. Multivariable linear regression models were adjusted for air pollutants (O₃ and PM_{2.5}), age, sex, African ancestry, number of household smokers, recruitment season, and maternal education.

* p-value <0.05

Text E1. Study Population

The Study of African Americans, Asthma, Genes & Environments (SAGE) is a case-control study of asthma among African Americans from the San Francisco Bay Area.^{E1} It was initiated in 2006 to examine the complex genetic and socio-environmental underpinnings of asthma among minority children and adolescents. Participants were aged 8–21 years and were recruited from multiple clinics and community health centers. Asthma was defined by a report of physician diagnosis and symptoms in the last 2 years. Participants were 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 of participating centers approved the study and all participants (or parents of participants younger than age 18 years) provided written informed consent. Full details of the SAGE study protocols have been described in detail elsewhere.^{E1-3} SAGE enrolled 1,710 participants (1,021 cases and 688 controls) from 2006 to August 2014.

Text E2. Determination of absolute telomere length***Measurement of Telomere Length***

Absolute telomere length (aTL) from PBMC was measured by determining the number of TTAGGG hexamer repeats per genome using quantitative real-time polymerase chain reaction (qPCR) as previously described.^{E4} This method is based on the qRT-PCR technique first described by Cawthon et al to measure relative telomere length (rTL) but modified to include: 1.) a telomeric and a single copy gene (*36B4*) standard curve and 2.) a commercially available DNA derived from a cell line to serve as positive control.^{E4} It has been suggested that the introduction of these two modifications increases the reproducibility of results between experimental assays and across labs as compared to rTL measurements.^{E4} In their publication, the authors demonstrated a strong correlation between their qPCR-based telomere length protocol and terminal restriction fragment analysis (TRF) by Southern hybridization).

Quantitative Polymerase Chain Reaction (qPCR) Protocol for Absolute Telomere Length

All samples were run on an ABI 7900HT Fast Real-Time PCR System running on the SDS Ver. 2.4 software (Applied Biosystems, Foster City, CA, USA). Each sample was analyzed in triplicate and accepted only if the standard deviation of the cycle threshold (C_T) values were $< 1 C_T$; accepted C_T values were then averaged for each sample. After assessing C_T values using this metric, 114 samples were removed from further analysis.

Each 20 μ l qPCR reaction master mix contained: 20 ng DNA, 1 $\times$ AmpliTaq Gold 360 Master Mix (Applied Biosystems, Foster City, CA), 10uM forward and reverse primers (Integrated DNA Technologies) for either telomere sequence or *36B4*, 1X *SybrGreen*. Cycling conditions (for both telomere and *36B4* products) were: 10 min at 95°C, followed by 40 cycles of 95°C, for 15 s and 60°C for 1 min. Serial dilutions of commercially available oligomers were used to

create telomere and 36B4 standard curves as previously described (O'Callaghan 2011)(Supplemental Methods Table 1).^{E4} DNA from the 1301 lymphoblastic cell line (ECACC 01051619) was used as a positive control and a no template water control was included in each qPCR assay. The inter- and intra-experimental coefficient of variation of the control DNA was 3% and 4.25%, respectively. Both the inter- and intra- experimental coefficient of variation were based on exponentiated qPCR data. Average amplification efficiency across plates was $\geq 90\%$ for both telomere and 36B4 assays. After all quality control procedures, aTL was computed for a total of 1599 (965 asthma cases and 634 healthy controls) SAGE II study participants.

Calculation of Absolute Telomere Length (aTL)

After amplification was complete, the ABI 7900 software produced values for each reaction that was equivalent to kb/reaction (calculated from the telomere C_T and standard curve) and diploid genome copy number/reaction (calculated from the 36B4 C_T and standard curve). The telomere kb per reaction value was divided by diploid genome copy number (calculated from the 36B4 C_T and standard curve) to give a total telomeric sequence length in kb per human diploid genome (absolute telomere length measurement).

Text E3. Assessment of ambient air pollution exposure

We used the inverse distance-squared weighted average from the four closest air pollution monitoring stations within 50 km of the participant's geocoded residence.^{E1,5} Ambient air pollution data were acquired from the U.S. Environmental Protection Agency Air Quality System (AQS). The exposures of those who moved during the course of a year were weighted based on the number of months spent at each residence.

Each address in a participant's residential history was geocoded using TomTom/Tele Atlas EZ-Locate software (TomTom, Amsterdam, the Netherlands). A "leave-one-out" evaluation of the assignment methodology using the AQS data from the four regions in 2006-2011 shows that monthly ozone concentrations are estimated within ± 2.5 ppb and $\pm 13\%$ error on average with an R^2 of 0.77. Monthly $PM_{2.5}$ concentrations are estimated with ± 1.5 $\mu g/m^3$ and $\pm 13\%$ error on average with an R^2 of 0.52. The mean bias for monthly ozone and $PM_{2.5}$ concentrations are -0.2 ppb or -1% and 0.2 $\mu g/m^3$ or 2%, respectively. The bias and error in the annual average values used for this analysis are similar or smaller than those for the month estimates.

Text E4. Variables or covariates selection

Selection of variables that might confound the association between air pollution and absolute TL was based on prior knowledge of known or suspected relations between exposure and covariates as well as the few published studies of air pollution and TL.^{E6-9} The covariates selected for inclusion in the final regression models were baseline age; sex; African ancestry (%; Text E6); asthma status (0=control, 1=case); adult smokers currently living with the participant; recruitment season (spring, summer, fall, winter); and maternal education (less than high school, high school, greater than high school) , which was used as a proxy for socioeconomic status (SES). We also adjusted for plate ID as a covariate to control for plate variation.

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Table E1. Summary of baseline characteristics of included versus not included SAGE study participants.

Characteristic	Included (N = 1,072)	Not included (N = 638)	P-value
Age, mean (SD), yrs [¶]	14.4 (3.6)	15.2 (4.0)	4.6e-05
N missing	0	0	
Male, N (%) [†]	535 (50.0)	284 (44.5)	0.03
N missing	0	0	
% African ancestry, mean (SD) [¶]	77.6 (12.3)	79.0 (11.3)	0.03
N missing	0	70	
Asthma cases, N (%) [†]	664 (62.0)	280 (43.9)	5.6e-13
N missing	0	0	
Current smokers in household, N (%) [†]			
None	726 (67.7)	171 (75.3)	0.03
One	240 (22.4)	43 (18.9)	0.3
Two or more	106 (9.9)	13 (5.7)	0.06
N missing	0	411	
Recruitment season, N (%) [†]			
Spring	271 (25.3)	44 (21.0)	0.2
Summer	416 (38.8)	83 (39.5)	0.9
Fall	165 (15.4)	35 (16.7)	0.7
Winter	220 (20.5)	48 (22.9)	0.5
N missing	0	428	
Maternal education, N (%) [†]			
< High school	142 (13.2)	117 (19.4)	0.001
= High school	293 (27.3)	170 (28.2)	0.7
> High school	637 (59.4)	316 (52.4)	0.006
N missing	0	35	

[¶]Unpaired two-samples t-test was performed for continuous variables.

[†]Two proportions z-test was applied for categorical variables.

The proportions may not sum to 100% due to round-up error.

Table E2. Average absolute TL (aTL) and air pollutant levels by recruitment season.

	Spring (N = 271)	Summer (N = 416)	Fall (N = 165)	Winter (N = 220)
aTL, kb	2,454.8 ± 1,223.7	2,498.6 ± 1,229.8	2,592.7 ± 1,489.3	2,442.1 ± 1,201.1
Age, yrs	14.0 ± 3.4	14.7 ± 3.7	14.5 ± 3.7	14.4 ± 3.7
O ₃ , ppb	20.5 ± 3.6	23.6 ± 3.3	22.7 ± 3.5	18.3 ± 3.8
PM _{2.5} , µg/m ³	8.5 ± 1.4	9.4 ± 1.3	8.8 ± 2.1	10.3 ± 2.1

Abbreviations: ppb, parts per billion

All values reported as mean ± SD

Table E3. Interaction between exposure to air pollution and asthma status on absolute TL.

Annual average (N = 1,072)	aTL [95% CI], kb	P-value
O ₃ × asthma status	32.8 [-18.4; 84.1]	0.21
PM _{2.5} × asthma status	36.9 [-79.3; 153.1]	0.53

O₃: ambient ozone. **PM_{2.5}**: particulate matter less than 2.5 µm in diameter. **pbb**: parts per billion. **µg/m³**: micrograms per cubic meter.

Estimates provided as a change in mean absolute telomere length (kb/diploid) for 1-ppb and 1-µg/m³ increases in O₃ and PM_{2.5}, respectively, for annual average (past year) exposures. Multivariable linear regression models were adjusted for air pollutants (O₃ and PM_{2.5}), age, sex, African ancestry, asthma status, number of household smokers, recruitment season, maternal education, and air pollutant × asthma status interaction term.

*p-value <0.05

Table E4. Association between absolute telomere length and annual average exposures to O₃ and PM_{2.5} among the asthma cases: 2006 -2014.

Exposure	aTL [95% CI], kb (N=645)	
	Base models <u>unadjusted</u> for duration of asthma	Base models <u>adjusted</u> for duration of asthma
O ₃	-9.0 [-40.1 to 22.0]	-8.6 [-39.4 to 22.2]
PM _{2.5}	-5.7 [-63.2 to 51.8]	-4.6 [-61.6 to 52.5]

Base models adjusted for air pollutants (O₃ and PM_{2.5}), age, sex, African ancestry, number of household smokers, recruitment season, and maternal education.

Table E5. Associations between covariates and absolute TL in SAGE study participants.

Variable (N=1,072)	aTL [95% CI][†], kb	aTL [95% CI][‡], kb
Age	-15.8 [-32.5; 0.9]	-26.1 [-52.3; 0.1]
Sex		
<i>Female (referent)</i>	1.0	1.0
<i>Male</i>	-52.2 [-170.8; 66.3]	-71.1 [-257.3; 115.1]
African ancestry proportion	5.9 [1.1; 10.7] [*]	8.4 [0.8; 15.9] [*]
Asthma status		
<i>Control (referent)</i>	1.0	1.0
<i>Case</i>	80.7 [-46.3; 207.8]	121.2 [-78.5; 320.9]
Smoking		
<i>None (referent)</i>	1.0	1.0
<i>One</i>	29.4 [-114.4; 173.2]	25.3 [-201.0; 251.6]
<i>Two or more</i>	160.0 [-41.6; 361.6]	229.2 [-87.6; 546.0]
Recruitment season		
<i>Fall (referent)</i>	1.0	1.0
<i>Spring</i>	-61.6 [-251.5; 128.3]	-133.6 [-437.5; 170.3]
<i>Summer</i>	-2.1 [-178.7; 174.5]	27.4 [-250.8; 305.6]
<i>Winter</i>	-77.7 [-276.5; 121.1]	-164.3 [-503.8; 175.2]
Maternal education		
<i>> High school (referent)</i>	1.0	1.0
<i>< High school</i>	9.0 [-169.8; 187.9]	-43.9 [-328.5; 240.7]
<i>= High school</i>	-29.9 [-166.4; 106.6]	-67.4 [-282.3; 147.5]

[†]Regression model was adjusted for age, sex, African ancestry (%), asthma status, number of household smokers, recruitment season, and maternal education.

[‡]Regression model was adjusted for annual average (past year) exposure to O₃ and PM_{2.5}, age, sex, African ancestry (%), asthma status, number of household smokers, recruitment season, and maternal education.

^{*}p-value <0.05

Table E6. Univariate analysis between age and age-group-specific mean TL (raw qPCR values).

Figure E2 (8 to 21 yrs)			Figure E3 (8 to 41 yrs)*		
	Estimate	P-value		Estimate	P-value
Age	-0.012	0.01	Age	-0.009	0.0008

*In Figure E3, we included older participants from SAGE I study, which yielded a wider age range (8 to 41 years).

Table E7. Oligomers used for aTL assay.

	Oligomer Name	Oligomer sequence (5'-3')
Standards	Telomere	(TTAGGG) ₁₄
	<i>36B4</i>	CAGCAAGTGGGAAGGTGTAATCCGTCTCCACAGACAAGGCC AGGACTCGTTTG
PCR Primers	teloF	CGGTTTGGTTTGGGTTTGGGTTTGGGTTTGGG TTTGGGTT
	teloR	GGCTTGCCTTACCCTTACCCTTACCC TTACCCTTACCCT
	<i>36B4F</i>	CAGCAAGTGGGAAGGTGTAATCC
	<i>36B4R</i>	CCCATTCTATCATCAACGGGTACAA

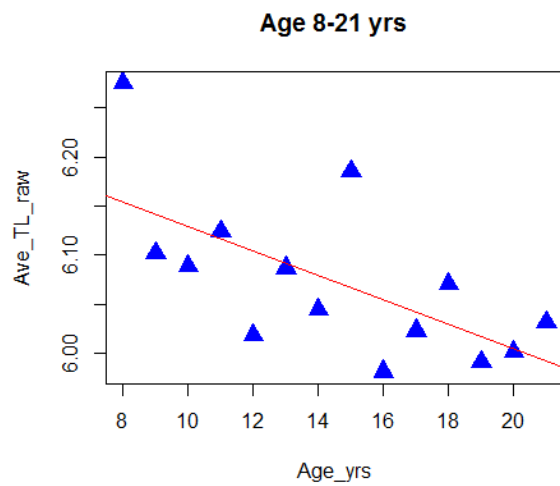


Figure E1. Relationship between TL (raw qPCR value) and age (8 to 21 yrs). The least squares linear regression line is presenting age on x-axis and age-group-specific mean TL (raw qPCR values) on y-axis.

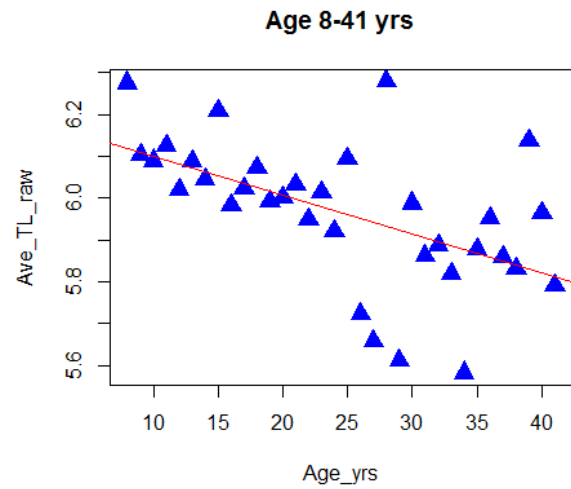


Figure E2. Relationship between TL (raw qPCR value) and age (8 to 41 yrs). The least squares linear regression line is presenting age on x-axis and age-group-specific mean TL (raw qPCR values) on y-axis. Older participants from SAGE I study was included, which yielded a wider age range.