Title

Ambient air pollution, asthma drug response, and telomere length in African American youth

Journal

Journal of Allergy and Clinical Immunology, 144(3)

ISSN

0091-6749

Authors

Lee, Eunice Y

Oh, Sam S

White, Marquitta J

et al.

Publication Date

2019-09-01

DOI

10.1016/j.jaci.2019.06.009

Peer reviewed

HHS Public Access

Author manuscript

J Allergy Clin Immunol. Author manuscript; available in PMC 2020 September 01.

Published in final edited form as:

J Allergy Clin Immunol. 2019 September ; 144(3): 839–845.e10. doi:10.1016/j.jaci.2019.06.009.

Ambient air pollution, asthma drug response, and telomere length in African American youth

Eunice Y. Lee, PhD[a,b], **Sam S. Oh, PhD**[b], **Marquitta J. White, PhD**[b], **Celeste S. Eng, BS**[b], **Jennifer R. Elhawary, BS**[b], **Luisa N. Borrell, DDS, PhD**[c], **Thomas J. Nuckton, MS, MD**[b], **Andrew M. Zeiger, BS**[b], **Kevin L. Keys, PhD**[b], **Angel C. Y. Mak, PhD**[b], **Donglei Hu, PhD**[b], **Scott Huntsman, MS**[b], **Maria G. Contreras, BS**[b,d], **Lesly-Anne Samedy, PharmD**[a], **Pagé C. Goddard, BS**[b], **Sandra L. Salazar, BS**[b], **Emerita N. Brigino-Buenaventura, MD**[e], **Adam Davis, MA, MPH**[f], **Kelley E. Meade, MD**[f], **Michael A. LeNoir, MD**[g], **Fred W. Lurmann, MS**[h], **Esteban G. Burchard, MD, MPH**[a,b,*], **Ellen A. Eisen, PhD**[i,*], **John R. Balmes, MD**[b,i,*]

[a]Department of Bioengineering & Therapeutic Sciences, University of California, San Francisco; [b]Department of Medicine, University of California, San Francisco; [c]Graduate School of Public Health & Health Policy, City University of New York; [d]San Francisco State University, San Francisco; [e]The Department of Allergy & Immunology, Kaiser Permanente-Vallejo Medical Center, Vallejo; [f]Children's Hospital and Research Center, Oakland; [g]Bay Area Pediatrics, Oakland; [h]Sonoma Technologies, Petaluma; [i]Environmental Health Sciences Division, School of Public Health, University of California, Berkeley.

Abstract

Background: Telomere length (TL) 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 TL, asthma, and air pollution is important for identifying risk factors contributing to unhealthy aging in children. Objectives: We sought to investigate associations between exposures to ambient air pollutants and 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 telomere length (aTL) and estimated annual average residential ozone (O_3) and fine particulate matter with a diameter of 2.5 μm or less ($PM_{2.5}$) exposures in a cross-sectional analysis of 1072 children in an existing asthma case-control study. African ancestry, asthma status, and use of steroid medications were examined as effect modifiers.

Corresponding author: Eunice Y. Lee, PhD, Department of Bioengineering and Therapeutic Sciences, Schools of Pharmacy and Medicine, UCSF, Rock Hall Box 2911, 1550 4th St, Rm 582, San Francisco, CA 94158. izeunice@gmail.com. Or: Esteban G. Burchard, MD, MPH, Department of Bioengineering and Therapeutic Sciences, Schools of Pharmacy and Medicine, UCSF, Rock Hall Box 2911, 1550 4th St, Rm 584B, San Francisco, CA 94158. esteban.burchard@ucsf.edu. Or: John R. Balmes, MD, San Francisco General Hospital Division of Occupational & Environmental Medicine, 1001 Potrero Ave, SFGH 30, San Francisco, CA 94143. john.balmes@ucsf.edu. Or: Ellen A. Eisen, PhD, School of Public Health, University of California, Berkeley, UCB, 751 University Hall, Berkeley, CA 94704. eeisen@berkeley.edu.

*These authors contributed equally to this work as shared senior authors.

Disclosure of potential conflict of interest: The authors declare that they have no relevant conflicts of interest.

Results: Participants' aTLs were measured by using quantitative PCR. A 1-ppb and 1 μg/m^3 increase in annual average exposure to O_3 and $PM_{2.5}$ were associated with a decrease in aTL of 37.1 kilo-base pair (kb; 95% CI, −66.7 to −7.4 kb) and 57.1 kb (95% CI, −118.1 to 3.9 kb), respectively. African ancestry and asthma were not effect modifiers; however, exposure to steroid medications modified the relationships between TL and pollutants. Past-year exposure to O_3 and $PM_{2.5}$ was associated with shorter TLs in patients without steroid use.

Conclusion: Exposure to air pollution was associated with shorter TLs in nonasthmatic children and adolescents. This was not the case for asthmatic children as a group, but those receiving steroid medication had less shortening than those not using steroids. Reduced exposure to air pollution in childhood might help to preserve TL.

Keywords

Ozone; fine particulate matter with a diameter of 2.5 μm or less; telomeres; asthma; minority; children

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 that cause oxidative stress in the airways.[11] This process could substantially affect TL because the high guanine content in telomeres confers sensitivity to reactive oxygen species.[11,12] Additionally, asthma is associated with increased oxidative stress in the airways, which can be aggravated by exposure to air pollution, 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 populations,[5–10] and information pertaining to minority children remains limited. Currently, there are 2 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 American subjects 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 effect of air pollution exposure on TL during childhood. Telomere damage incurred while *in utero*, in childhood,

or both might 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 persons of 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. Although the factors captured by these categories contribute to clinical practice and biomedical research, use of race/ethnicity is widely debated. TL 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 American subjects in addition to self-identified race/ethnicity.

Here we used case-control data to examine the association between TL and long-term average daily exposures to ozone (O_3) and fine particulate matter with a diameter of 2.5 μm or less ($PM_{2.5}$) 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 TL 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, TL, and covariates specified below in the statistical analysis. After excluding 638 subjects with missing data, a total of 1072 subjects (aged 8–21 years), both asthmatic patients and control subjects, were used in this analysis (Table I). Eligibility required all participants to self-identify as African American. In addition, all 4 biologic grandparents were required to be identified as African American. Information about SAGE and subject selection criteria are outlined in the Methods section in this article's Online Repository at www.jacionline.org, and descriptive statistics for subjects included and excluded in the analysis are summarized in Table E1 in this article's Online Repository at www.jacionline.org.

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

Determination of absolute TL

Genomic DNA was isolated from whole blood, according to the manufacturer's recommendation, by using Wizard Genomic DNA Purification Kits (Promega, Fitchburg, Wis). Genomic DNA was assessed by using agarose gel for DNA degradation; all samples demonstrated uniform intensity (>10 kilo-base pair [kb]), and no smears were observed, indicating intact DNA samples. A NanoDrop ND-1000 spectrophotometer (Thermo Scientific, Waltham, Mass) was used to assess the quality and quantity of extracted DNA. All samples assayed had absorbance ratios (260/280) of between 1.8 and 2.0. Absolute telomere length (aTL) was measured in triplicate by determining the number of TTAGGG hexamer repeats by using quantitative real-time PCR (qPCR), as previously described (see the Methods section in this article's Online Repository).[34] Use of the qPCR method is a widespread practice in air pollution, asthma, and TL association studies because of its ability to generate high-throughput TL data.[35,36] More information about aTL measurement can be found in the Methods section in this article's Online Repository.

Assessment of ambient air pollution exposure

We focused on O_3 and $PM_{2.5}$ as the main air pollution exposures because of their ability to form free radicals and cause oxidative damage.[11] Concentrations of ambient O_3 and $PM_{2.5}$ were predicted by air pollution exposure models at the individual level. Each participant's exposure to ambient levels of O_3 (in parts per billion) and $PM_{2.5}$ (in micrograms per cubic meter) was assessed based on geocoded residential addresses at the point of enrollment and air quality data from regional monitoring stations, as previously described (see the Methods section in this article's Online Repository).[28,37] We defined 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, Calif), which includes 817,810 single nucleotide polymorphisms 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 with ADMIXTURE assuming 2 ancestral populations.[39] Genotype data from European (CEU) and African (YRI) samples from HapMap phase II were included as a reference.[40] African ancestry was converted to represent a percentage and considered as a potential confounder and effect modifier.

Statistical analysis

Characteristics for SAGE participants were summarized by using means (SDs) for continuous variables and frequencies (proportions) for categorical variables. Linear regression was used to model aTL 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 the Methods section in this article's Online

Repository. Consistent with previous studies, O_3 and $PM_{2.5}$ exposure variables were both included in each model.[28,37] Correlation between past year O_3 and $PM_{2.5}$ exposures was −0.55.

African ancestry, asthma status, and steroid medication use were also considered potential effect modifiers. For each potential modifier, an interaction term was added to the regression model to test statistical significance: African ancestry (0, less than median African ancestry; 1, greater than median African ancestry), asthma status (0, control; 1, case), inhaled corticosteroid (ICS) use (0, no; 1, yes), and any steroid use (ICSs, oral steroids, or both) in the past year (0, no; 1, yes). 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 aTL was treated as a continuous variable, and the range of aTLs varied from 400 to 8693 kb. Because aTL was not normally distributed, we performed analyses on the log-transformed aTL value, which passed the Shapiro-Wilk test for normality. Effect estimates were back-transformed and reported as change in mean aTL (kb/diploid).

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

RESULTS

Characteristics of the study population

Demographic characteristics of the 1072 study participants are shown in Table I. Mean age was 14.4 ± 3.6 years and ranged from 8 to 21 years. Average genetic African ancestry was 77.6% ± 12.3%. There were more asthma cases (62.0%) than noncases. Approximately 59.4% of the study subjects' mothers obtained a high level of education. In SAGE participants mean aTL was 2490.4 ± 1265.1 kb. Subjects included in the analysis were more likely to be male and asthmatic and have a lower African ancestry score than those excluded because of incomplete data (see Table E1). Average aTL was similar across recruitment seasons, although participants recruited in the fall had modestly longer telomeres (see Table E2 in this article's Online Repository at www.jacionline.org). Pollutant levels were slightly greater in the summer (see Table E2).

Associations between long-term exposures to ambient air pollutants and aTL

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

Asthma was not a significant effect modifier for air pollution exposure and TL (see Table E3 in this article's Online Repository at www.jacionline.org). The effect of past-year exposure to O_3 and $PM_{2.5}$ on TL was significant only in participants without asthma (Table III). aTL

decreased by 89.5 and 206.2 kb with every 1-ppb and 1 μg/m^3 increase in O_3 and $PM_{2.5}$ exposure levels, respectively (95% CI_{O3}, −147.7 to −31.2 kb; 95% $CI_{PM2.5}$, −348.8 to −63.7 kb).

We observed that steroid medication use interacted with $PM_{2.5}$ exposures among asthma cases (Table IV). Additive associations of interaction terms suggest that ICS use resulted in a 164.1-kb (95% CI, 18.6 to 309.6 kb) longer TL with a 1 μg/m^3 increase in $PM_{2.5}$ exposure. Use of any steroid in the last 12 months was also associated with a 175.9-kb (95% CI, −1.3 to 353.0 kb) longer TL at a marginally significant level (P =.06) with a 1 μg/m^3 increase in $PM_{2.5}$. Results for interactions between O_3 exposure and steroid use were null.

In stratified analyses (Table V) the magnitude and direction of associations varied by the type of steroid medication used. Although the results for ICS and any steroid use were not statistically significant, general trends of association were toward a longer TL. Among non-ICS users, a 1-ppb and a 1 μg/m^3 increase in past-year exposure to O_3 and $PM_{2.5}$ were significantly associated with a 86.0- and 190.9-kb decrease in TL, respectively (95% CI_{O3}, −171.0 to −1.0 kb; 95% $CI_{PM2.5}$, −374.3 to −7.4 kb). The effects of air pollution exposure on TL were the greatest in magnitude among the asthmatic patients without any history of steroid use. Annual average exposure to O_3 and $PM_{2.5}$ decreased aTL by 123.5 and 314.9 kb, respectively (95% CI_{O3}, −248.4 to 1.4 kb; 95% $CI_{PM2.5}$, −594.2 to −35.7 kb) with every 1-ppb and 1 μg/m^3 increase in O_3 and $PM_{2.5}$ exposure levels, respectively.

We also considered adjusting for the duration of asthma (in years); however, inclusion of that term did not affect any of the associations between past-year exposures to air pollutants and TLs among the asthma cases (see Table E4 in this article's Online Repository at www.jacionline.org).

Association between African ancestry and aTL

After adjusting for covariates, a 1% increase in African genetic ancestry was associated with an 8.4-kb longer aTL (95% CI, 0.8 to 15.9 kb; Table II). 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 American subjects during childhood and adolescence might increase the risk of telomere shortening. For example, we found that the annual average exposure to O_3 was associated with a shorter aTL. Considering that 1 year of aging was associated with 15.8- to 26.1-kb decreases in aTLs (see Table E5 in this article's Online Repository at www.jacionline.org), a 1-ppb increase in O_3 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 asthmatic patients revealed that use of ICSs 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 O_3 and $PM_{2.5}$ were associated with decreased aTL, 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 surfaces of fine particulate matter can induce generation of free radicals in the airways, which can cause telomere shortening through an oxidative stress mechanism.[11,43,44] Certain bioaerosols, 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 concentrations, 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 can 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 aTL is further explored below.

Associations between exposure to air pollution and aTL were significant only among the nonasthmatic participants, and magnitudes were stronger than among asthmatic patients. This finding was counterintuitive to our initial hypothesis. However, when we stratified asthma cases by use of inhaled steroid or any steroid use, past-year exposure to O_3 and $PM_{2.5}$ resulted in significantly shorter TLs among non-ICS users. Moreover, associations of O_3 and $PM_{2.5}$ were greatest in magnitude among the asthmatic patients who reported no steroid use over the last 12 months. The interaction results shown in Table IV suggest that a 1 $\mu g/m^3$ increase in $PM_{2.5}$ exposure is associated with a significant increase in the aTL of 164.1 kb in asthmatic patients using ICSs. Positive interactions between $PM_{2.5}$ and steroid 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 asthma severity, plays a role. The exact mechanism of telomere elongation with steroid medication use is not clear and beyond the scope of this article.

Although not a study of the effect of corticosteroids, Townsley et al[48] have reported that androgen anabolic steroid therapy was effective in reducing telomere loss and lengthening telomeres, possibly mediated by upregulation of telomerase activities, in patients with genetically defective telomeres. One possible mechanism by which ICSs could attenuate the effect of air pollution on TL in PBMCs 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 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]

Varying levels of African, European, and Native American ancestry in African American subjects 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 have 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 American and Latino subjects.[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% increase in African ancestry was associated with a 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 aTL. One possible explanation is that the negatively skewed distribution of African ancestry (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 and progenitor cells that form peripheral leukocytes. On average, subjects of African descent have a lower number of circulating neutrophils than subjects of white descent. Longer TLs in African American children than in white children are likely a result of fewer replications of hematopoietic stem cells/progenitor cells.[29]

We observed an inverse association between TL and age (Table II). However, it might require a wider age range than our study to observe a significant age effect (see Figs E1 and E2 and Table E6 in this article's Online Repository at www.jacionline.org).

Strengths and limitations

Our study is one of the very few that has investigated the relationship between exposure to air pollution and aTL in children and adolescents living in the United States. We studied a relatively large population of well-characterized participants using established methods. aTL 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 aTL. Average levels of past-year exposure to O_3 and $PM_{2.5}$ in our study were less than US national ambient air quality standards. Our study results suggest that even relatively low-level air pollution can adversely affect aTLs 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 aTLs 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. Although our exposure assessment is widely used in large-scale air pollution epidemiologic studies,[53–55] exposures of individual subjects can be underestimated or overestimated because 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 nondifferential and therefore lead to underestimation of the true effect size. Integrating personal air quality monitors/sensors with air pollution exposure models would be the most ideal method to accurately measure the exposure levels to ambient and indoor air pollution. However, 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 logistical issues entailed by such monitoring. For future studies, incorporating time-activity patterns or using personal

monitors to measure air quality at least in a subset of participants might improve exposure assessment.

Because our study used a cross-sectional analysis, we cannot make a 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 ICSs on the air pollution–TL relationship would improve our understanding.

This study is the first to investigate the association between exposure to ambient air pollution and aTLs in African American children and adolescents living in an urban setting. We discovered that 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 ICSs, long-term treatment, among children with asthma. We found that annual average exposures 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 could improve TL maintenance, which might be associated with improved health outcomes in African American children and adolescents.

METHODS

Study population

SAGE is a case-control study of asthma among African American subjects from the San Francisco Bay Area.[E1] It was initiated in 2006 to examine the complex genetic and socioenvironmental underpinnings of asthma among minority children and adolescents. Participants were aged 8 to 21 years and recruited from multiple clinics and community health centers. Asthma was defined by report of a physician's 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 of age) provided written informed consent. Full details of SAGE protocols have been described in detail elsewhere.[E1–E3] SAGE enrolled 1709 participants (1021 cases and 688 control subjects) from 2006 to August 2014.

Determination of aTL

Measurement of TL.—aTLs from PBMCs were measured by determining the number of TTAGGG hexamer repeats per genome with qPCR, as previously described.[E4] This method is based on the quantitative RTPCR technique first described by Cawthon et al[E5] to measure relative TL but modified to include the following: (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 introduction of these 2 modifications increases the reproducibility of results between experimental assays and across laboratories compared with relative TL measurements.[E4] In their publication the authors demonstrated a

strong correlation between their qPCR-based TL protocol and terminal restriction fragment analysis by using Southern hybridization).

qPCR protocol for aTL.—All samples were run on an ABI 7900HT Fast Real-Time PCR System running on SDS software (version 2.4; Applied Biosystems, Foster City, Calif). Each sample was analyzed in triplicates and accepted only if the SD of the cycle threshold (C_T) values were less than 1 C_T; accepted C_T values were then averaged for each sample. After assessing C_T values by using this metric, 114 samples were removed from further analysis.

Each 20-μL qPCR reaction master mix contained the following: 20 ng of DNA, 1 × AmpliTaq Gold 360 Master Mix (Applied Biosystems), and 10 μmol/L forward and reverse primers (see Table E7; Integrated DNATechnologies, Coralville, Iowa) for either telomere sequence or *36B4* 1 × SYBR Green. Cycling conditions (for both telomere and *36B4* products) were as follows: 10 minutes at 95°C, followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. Serial dilutions of commercially available oligomers were used to create telomere and *36B4* standard curves, as previously described (Table E1).[E4] DNA from the 1301 lymphoblastic cell line (ECACC01051619) was used as a positive control, and a no-template water control was included in each qPCR assay. The interexperimental and intraexperimental coefficient of variation of the control DNA was 3% and 4.25%, respectively. Both interexperimental and intraexperimental coefficients of variation were based on exponentiated qPCR data. Average amplification efficiency across plates was 90% or greater for both the telomere and *36B4* assays. After all quality control procedures, aTLs were computed for a total of 1599 (965 asthmatic patients and 634 healthy control subjects) SAGE II study participants.

Calculation of aTL.—After amplification was complete, the ABI 7900 software produced values for each reaction that was equivalent to kb per reaction (calculated from the telomere C_T and standard curve) and diploid genome copy number per 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 produce a total telomeric sequence length in kb per human diploid genome (aTL measurement).

Assessment of ambient air pollution exposure

We used the inverse distance-squared weighted average from the 4 closest air pollution monitoring stations within 50 km of the participant's geocoded residence.[E1,E6] Ambient air pollution data were acquired from the US Environmental Protection Agency's Air Quality System. 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 by using TomTom/Tele Atlas EZ-Locate software (TomTom, Amsterdam, The Netherlands). A "leave-one-out" evaluation of the assignment methodology using Air Quality System data from the 4 regions in 2006–2011 shows that monthly O_3 concentrations are estimated to be within ±2.5 ppb and ±13% error on average, with an R^2 value of 0.77. Monthly $PM_{2.5}$ concentrations are estimated to be within ±1.5 μg/m^3 and ±13% error on average, with an R^2 value of 0.52. The mean biases for monthly O_3 and $PM_{2.5}$ concentrations are −0.2 ppb or −1% and 0.2 μg/m^3

or 2%, respectively. Biases and errors in annual average values used for this analysis are similar or smaller than those for month estimates.

Variables or covariate selection

Selection of variables that might confound the association between air pollution and aTL 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.[E7–E10] Covariates selected for inclusion in the final regression models were baseline age, sex, African ancestry (percentage), asthma status (0, control; 1, case), adult smokers currently living with the participant, recruitment season (spring, summer, fall, and winter), and maternal education (less than high school, high school, and greater than high school), which was used as a proxy for socioeconomic status. We also adjusted for plate ID as a covariate to control for plate variation.

Acknowledgments

Supported in part by the Sandler Family Foundation; the American Asthma Foundation; the RWJF Amos Medical Faculty Development Program; the Harry Wm. and Diana V. Hind Distinguished Professorship in Pharmaceutical Sciences II; the National Institute of Health; General Medical Sciences grant T32GM007546; National Institutes of Health grants R01HL117004, R01HL128439, R01HL135156, 1X01HL134589, and R01HL141992; National Institute of Health and Environmental Health Sciences grants R01ES015794 and R21ES24844; National Institute on Minority Health and Health Disparities grants P60MD006902 RL5GM118984, and R01MD010443; the Tobacco-Related Disease Research Program under award no. 27IR-0030; and National Institute for Occupational Health and Safety grant T42 OH008429.

Abbreviations used

aTL	Absolute telomere rate
C_T	Cycle threshold
ICS	Inhaled corticosteroid
kb	Kilo-base pair
O_3	Ozone
$PM_{2.5}$	Fine particulate matter with a diameter of 2.5 μm or less
qPCR	Quantitative real-time PCR
SAGE	Study of African Americans, Asthma, Genes & Environments
TL	Telomere length

REFERENCES

1. Blackburn EH. Structure and function of telomeres. Nature 1991;350:569–73. [PubMed: 1708110]
2. Blackburn EH. Telomere states and cell fates. Nature 2000;408:53–6. [PubMed: 11081503]
3. Blackburn EH, Epel ES, Lin J. Human telomere biology: a contributory and interactive factor in aging, disease risks, and protection. Science 2015;350:1193–8. [PubMed: 26785477]

4. Fitzpatrick AL, Kronmal RA, Kimura M, Gardner JP, Psaty BM, Jenny NS, et al. Leukocyte telomere length and mortality in the Cardiovascular Health Study. J Gerontol A Biol Sci Med Sci 2011;66:421–9. [PubMed: 21289018]
5. Kimura M, Hjelmborg JV, Gardner JP, Bathum L, Brimacombe M, Lu X, et al. Telomere length and mortality: a study of leukocytes in elderly Danish twins. Am J Epidemiol 2008;167:799–806. [PubMed: 18270372]
6. Lee J, Sandford AJ, Connett JE, Yan J, Mui T, Li Y, et al. The relationship between telomere length and mortality in chronic obstructive pulmonary disease (COPD). PLoS One 2012;7:e35567. [PubMed: 22558169]
7. Sanders JL, Newman AB. Telomere length in epidemiology: a biomarker of aging, age-related disease, both, or neither? Epidemiol Rev 2013;35:112–31. [PubMed: 23302541]
8. Cassidy A, De Vivo I, Liu Y, Han J, Prescott J, Hunter DJ, et al. Associations between diet, lifestyle factors, and telomere length in women. Am J Clin Nutr 2010; 91:1273–80. [PubMed: 20219960]
9. Kiecolt-Glaser JK, Glaser R. Psychological stress, telomeres, and telomerase. Brain Behav Immun 2010;24:529–30. [PubMed: 20167271]
10. Mirabello L, Huang WY, Wong JY, Chatterjee N, Reding D, Crawford ED, et al. The association between leukocyte telomere length and cigarette smoking, dietary and physical variables, and risk of prostate cancer. Aging Cell 2009;8:405–13. [PubMed: 19493248]
11. Kelly FJ. Oxidative stress: its role in air pollution and adverse health effects. Occup Environ Med 2003;60:612–6. [PubMed: 12883027]
12. Oikawa S, Kawanishi S. Site-specific DNA damage at GGG sequence by oxidative stress may accelerate telomere shortening. FEBS Lett 1999;453:365–8. [PubMed: 10405177]
13. Liu L, Poon R, Chen L, Frescura AM, Montuschi P, Ciabattoni G, et al. Acute effects of air pollution on pulmonary function, airway inflammation, and oxidative stress in asthmatic children. Environ Health Perspect 2009;117:668–74. [PubMed: 19440509]
14. Guerra S New asthma biomarkers: shorter telomeres, longer disease? Am J Respir Crit Care Med 2014;190:356–8. [PubMed: 25127300]
15. Bijnens E, Zeegers MP, Gielen M, Kicinski M, Hageman GJ, Pachen D, et al. Lower placental telomere length may be attributed to maternal residential traffic exposure; a twin study. Environ Int 2015;79:1–7. [PubMed: 25756235]
16. Hou L, Wang S, Dou C, Zhang X, Yu Y, Zheng Y, et al. Air pollution exposure and telomere length in highly exposed subjects in Beijing, China: a repeated-measure study. Environ Int 2012;48:71–7. [PubMed: 22871507]
17. Hoxha M, Dioni L, Bonzini M, Pesatori AC, Fustinoni S, Cavallo D, et al. Association between leukocyte telomere shortening and exposure to traffic pollution: a cross-sectional study on traffic officers and indoor office workers. Environ Health 2009;8:41. [PubMed: 19772576]
18. Martens DS, Cox B, Janssen BG, Clemente DBP, Gasparrini A, Vanpoucke C, et al. Prenatal air pollution and newborns' predisposition to accelerated biological aging. JAMA Pediatr 2017;171:1160–7. [PubMed: 29049509]
19. Lee EY, Lin J, Noth EM, Hammond SK, Nadeau KC, Eisen EA, et al. Traffic-related air pollution and telomere length in children and adolescents living in Fresno, CA: a pilot study. J Occup Environ Med 2017;59:446–52. [PubMed: 28486341]
20. Walton RT, Mudway IS, Dundas I, Marlin N, Koh LC, Aitlhadj L, et al. Air pollution, ethnicity and telomere length in east London schoolchildren: an observational study. Environ Int 2016;96:41–7. [PubMed: 27591803]
21. Clark LP, Millet DB, Marshall JD. National patterns in environmental injustice and inequality: outdoor NO2 air pollution in the United States. PLoS One 2014;9: e94431. [PubMed: 24736569]
22. Fleischman L, Franklin M. Clean Air Task Force, NAACP. Fumes across the fence-line: the health impacts of air pollution from oil & gas facilities on African American communities. 2017 Available at: http://www.catf.us/wp-content/uploads/2017/11/CATF_Pub_FumesAcrossTheFenceLine.pdf. Accessed July 3, 2019.
23. Jones MR, Diez-Roux AV, Hajat A, Kershaw KN, O'Neill MS, Guallar E, et al. Race/ethnicity, residential segregation, and exposure to ambient air pollution: the Multi-Ethnic Study of Atherosclerosis (MESA). Am J Public Health 2014;104: 2130–7. [PubMed: 25211756]

24. Mikati I, Benson AF, Luben TJ, Sacks JD, Richmond-Bryant J. Disparities in distribution of particulate matter emission sources by race and poverty status. Am J Public Health 2018;108:480–5. [PubMed: 29470121]
25. Morello-Frosch R, Jesdale BM. Separate and unequal: residential segregation and estimated cancer risks associated with ambient air toxics in U.S. metropolitan areas. Environ Health Perspect 2006;114:386–93. [PubMed: 16507462]
26. Morello-Frosch R, Shenassa ED. The environmental "riskscape" and social inequality: implications for explaining maternal and child health disparities. Environ Health Perspect 2006;114:1150–3. [PubMed: 16882517]
27. Parsa A1, Kao WH, Xie D, Astor BC, Li M, Hsu CY, et al. APOL1 risk variants, race, and progression of chronic kidney disease. N Engl J Med 2013;369: 2183–96. [PubMed: 24206458]
28. Foreman MG, Zhang L, Murphy J, Hansel NN, Make B, Hokanson JE, et al. Early-onset chronic obstructive pulmonary disease is associated with female sex, maternal factors, and African American race in the COPDGene study. Am J Respir Crit Care Med 2011;184:414–20. [PubMed: 21562134]
29. Hunt SC, Chen W, Gardner JP, Kimura M, Srinivasan SR, Eckfeldt JH, et al. Leukocyte telomeres are longer in African Americans than in whites: the National Heart, Lung, and Blood Institute Family Heart Study and the Bogalusa Heart Study. Aging Cell 2008;7:451–8. [PubMed: 18462274]
30. Zhu H, Wang X, Gutin B, Davis CL, Keeton D, Thomas J, et al. Leukocyte telomere length in healthy Caucasian and African-American adolescents: relationships with race, sex, adiposity, adipokines, and physical activity. J Pediatr 2011;158: 215–20. [PubMed: 20855079]
31. Borrell LN, Nguyen EA, Roth LA, Oh SS, Tcheurekdjian H, Sen S, et al. Childhood obesity and asthma control in the GALA II and SAGE II studies. Am J Respir Crit Care Med 2013;187:697–702. [PubMed: 23392439]
32. Nishimura KK, Galanter JM, Roth LA, Oh SS, Thakur N, Nguyen EA, et al. Early-life air pollution and asthma risk in minority children. The GALA II and SAGE II studies. Am J Respir Crit Care Med 2013;188:309–18. [PubMed: 23750510]
33. Thakur N, Oh SS, Nguyen EA, Martin M, Roth LA, Galanter J, et al. Socioeconomic status and childhood asthma in urban minority youths. The GALA II and SAGE II studies. Am J Respir Crit Care Med 2013;188:1202–9. [PubMed: 24050698]
34. O'Callaghan NJ, Fenech M. A quantitative PCR method for measuring absolute telomere length. Biol Proced Online 2011;13:3. [PubMed: 21369534]
35. Albrecht E, Sillanpaa E, Karrasch S, Alves AC, Codd V, Hovatta I, et al. Telomere length in circulating leukocytes is associated with lung function and disease. Eur Respir J 2014;43:983–92. [PubMed: 24311771]
36. Zhao B, Vo HQ, Johnston FH, Negishi K. Air pollution and telomere length: a systematic review of 12,058 subjects. Cardiovasc Diagn Ther 2018;8:480–92. [PubMed: 30214863]
37. Neophytou AM, White MJ, Oh SS, Thakur N, Galanter JM, Nishimura KK, et al. Air pollution and lung function in minority youth with asthma in the GALA II (Genes-Environments and Admixture in Latino Americans) and SAGE II (Study of African Americans, Asthma, Genes, and Environments) studies. Am J Respir Crit Care Med 2016;193:1271–80. [PubMed: 26734713]
38. Hoffmann TJ, Zhan Y, Kvale MN, Hesselson SE, Gollub J, Iribarren C, et al. Design and coverage of high throughput genotyping arrays optimized for individuals of East Asian, African American, and Latino race/ethnicity using imputation and a novel hybrid SNP selection algorithm. Genomics 2011;98: 422–30. [PubMed: 21903159]
39. Alexander DH, Novembre J, Lange K. Fast model-based estimation of ancestry in unrelated individuals. Genome Res 2009;19:1655–64. [PubMed: 19648217]
40. International HapMap Consortium Altshuler DM, Gibbs RA, Peltonen L, Altshuler DM, Gibbs RA, et al. Integrating common and rare genetic variation in diverse human populations. Nature 2010;467:52–8. [PubMed: 20811451]
41. Bertolini V, Gandolfi I, Ambrosini R, Bestetti G, Innocente E, Rampazzo G, et al. Temporal variability and effect of environmental variables on airborne bacterial communities in an urban area of Northern Italy. Appl Microbiol Biotechnol 2013;97:6561–70. [PubMed: 23053100]

42. Putaud JP, Raes F, Van Dingenen R, Br€uggemann E, Facchini MC, Decesari S, et al. European aerosol phenomenology-2: chemical characteristics of particulate matter at kerbside, urban, rural and background sites in Europe. Atmos Environ 2004;38:2579–95.

43. Sorensen M, Schins RP, Hertel O, Loft S. Transition metals in personal samples of PM2.5 and oxidative stress in human volunteers. Cancer Epidemiol Biomarkers Prev 2005;14:1340–3. [PubMed: 15894700]

44. Xia T, Korge P, Weiss JN, Li N, Venkatesen MI, Sioutas C, et al. Quinones and aromatic chemical compounds in particulate matter induce mitochondrial dysfunction: implications for ultrafine particle toxicity. Environ Health Perspect 2004;112: 1347–58. [PubMed: 15471724]

45. Samake A, Uzu G, Martins JMF, Calas A, Vince E, Parat S, et al. The unexpected role of bioaerosols in the oxidative potential of PM. Sci Rep 2017;7: 10978. [PubMed: 28887459]

46. Perrone MG, Gualtieri M, Consonni V, Ferrero L, Sangiorgi G, Longhin E, et al. Particle size, chemical composition, seasons of the year and urban, rural or remote site origins as determinants of biological effects of particulate matter on pulmonary cells. Environ Pollut 2013;176:215–27. [PubMed: 23434772]

47. Perrone S, Negro S, Tataranno ML, Buonocore G. Oxidative stress and antioxidant strategies in newborns. J Matern Fetal Neonatal Med 2010;23(suppl 3):63–5. [PubMed: 20807155]

48. Townsley DM, Dumitriu B, Young NS. Danazol treatment for telomere diseases. N Engl J Med 2016;375:1095–6.

49. Delfino RJ, Zeiger RS, Seltzer JM, Street DH, McLaren CE. Association of asthma symptoms with peak particulate air pollution and effect modification by anti-inflammatory medication use. Environ Health Perspect 2002;110: A607–17. [PubMed: 12361942]

50. Qian Z, Lin HM, Chinchilli VM, Lehman EB, Duan Y, Craig TJ, et al. Interaction of ambient air pollution with asthma medication on exhaled nitric oxide among asthmatics. Arch Environ Occup Health 2009;64:168–76. [PubMed: 19864219]

51. Kumar R, Seibold MA, Aldrich MC, Williams LK, Reiner AP, Colangelo L, et al. Genetic ancestry in lung-function predictions. N Engl J Med 2010;363: 321–30. [PubMed: 20647190]

52. Pino-Yanes M, Thakur N, Gignoux CR, Galanter JM, Roth LA, Eng C, et al. Genetic ancestry influences asthma susceptibility and lung function among Latinos. J Allergy Clin Immunol 2014;135:228–35. [PubMed: 25301036]

53. Jerrett M, McConnell R, Wolch J, Chang R, Lam C, Dunton G, et al. Traffic-related air pollution and obesity formation in children: a longitudinal, multilevel analysis. Environ Health 2014;13:49. [PubMed: 24913018]

54. Pope CA 3rd, Turner MC, Burnett RT, Jerrett M, Gapstur SM, Diver WR, et al. Relationships between fine particulate air pollution, cardiometabolic disorders, and cardiovascular mortality. Circ Res 2015;116:108–15. [PubMed: 25348167]

55. Turner MC, Jerrett M, Pope CA 3rd, Krewski D, Gapstur SM, Diver WR, et al. Long-term ozone exposure and mortality in a large prospective study. Am J Respir Crit Care Med 2016;193:1134–42. [PubMed: 26680605]

References

E1. Nishimura KK, Galanter JM, Roth LA, Oh SS, Thakur N, Nguyen EA, et al. Early-life air pollution and asthma risk in minority children. The GALA II and SAGE II studies. Am J Respir Crit Care Med 2013;188:309–18. [PubMed: 23750510]

E2. Borrell LN, Nguyen EA, Roth LA, Oh SS, Tcheurekdjian H, Sen S, et al. Childhood obesity and asthma control in the GALA II and SAGE II studies. Am J Respir Crit Care Med 2013;187:697–702. [PubMed: 23392439]

E3. Thakur N, Oh SS, Nguyen EA, Martin M, Roth LA, Galanter J, et al. Socioeconomic status and childhood asthma in urban minority youths. The GALA II and SAGE II studies. Am J Respir Crit Care Med 2013;188:1202–9. [PubMed: 24050698]

E4. O'Callaghan NJ, Fenech M. A quantitative PCR method for measuring absolute telomere length. Biol Proced Online 2011;13:3. [PubMed: 21369534]

E5. Cawthon RM. Telomere measurement by quantitative PCR. Nucleic Acids Res 2002;30:e47. [PubMed: 12000852]

E6. Neophytou AM, White MJ, Oh SS, Thakur N, Galanter JM, Nishimura KK, et al. Air pollution and lung function in minority youth with asthma in the GALA II (Genes-Environments and Admixture in Latino Americans) and SAGE II (Study of African Americans, Asthma, Genes, and Environments) studies. Am J Respir Crit Care Med 2016;193:1271–80. [PubMed: 26734713]

E7. Hou L, Wang S, Dou C, Zhang X, Yu Y, Zheng Y, et al. Air pollution exposure and telomere length in highly exposed subjects in Beijing, China: a repeated-measure study. Environ Int 2012;48:71–7. [PubMed: 22871507]

E8. Hoxha M, Dioni L, Bonzini M, Pesatori AC, Fustinoni S, Cavallo D, et al. Association between leukocyte telomere shortening and exposure to traffic pollution: a cross-sectional study on traffic officers and indoor office workers. Environ Health 2009;8:41. [PubMed: 19772576]

E9. Martens DS, Cox B, Janssen BG, Clemente DBP, Gasparrini A, Vanpoucke C, et al. Prenatal air pollution and newborns' predisposition to accelerated biological aging. JAMA Pediatr 2017;171:1160–7. [PubMed: 29049509]

E10. Bijnens E, Zeegers MP, Gielen M, Kicinski M, Hageman GJ, Pachen D, et al. Lower placental telomere length may be attributed to maternal residential traffic exposure; a twin study. Environ Int 2015;79:1–7. [PubMed: 25756235]

Clinical implications:

Exposure to air pollution might be associated with shorter TLs in nonasthmatic children. Among asthmatic children, steroid medication use appeared to reduce the risk of telomere shortening associated with exposure to air pollution.

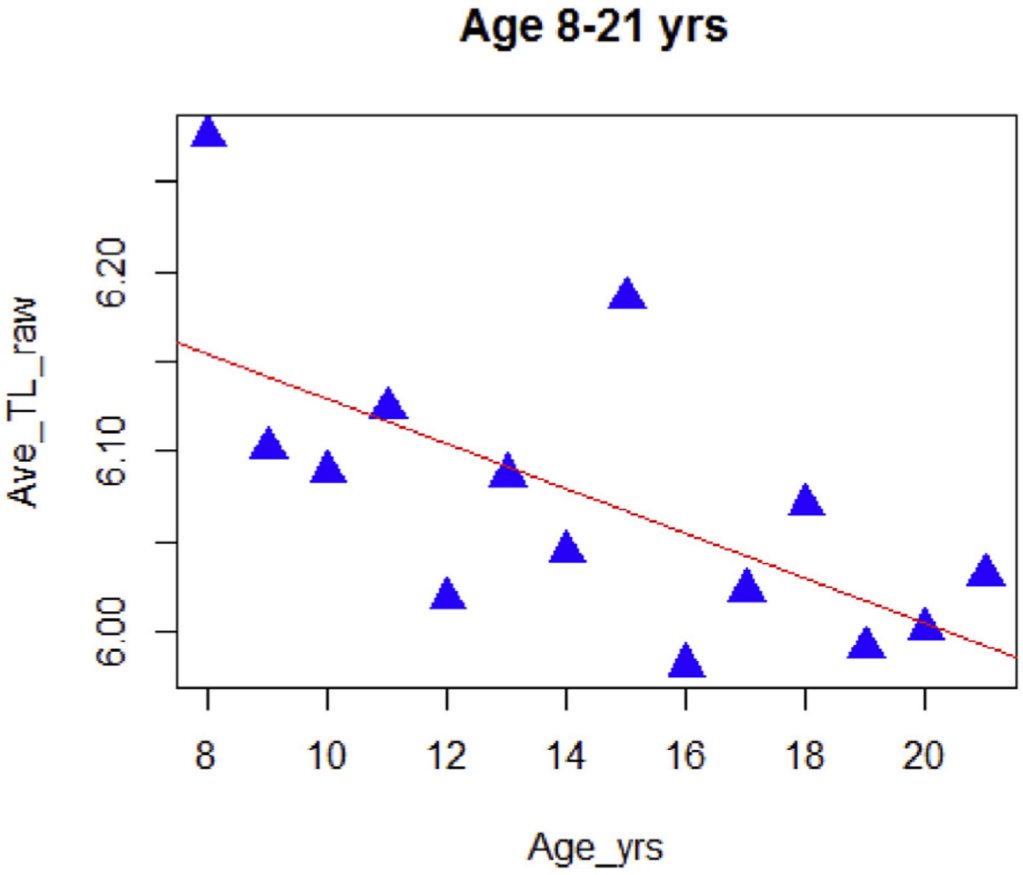

FIG E1.

Relationship between TL (raw qPCR value) and age (8 to 21 years). The *least squares linear regression line* presents age on the *x-axis* and age group–specific mean TL (raw qPCR values) on the *y-axis*.

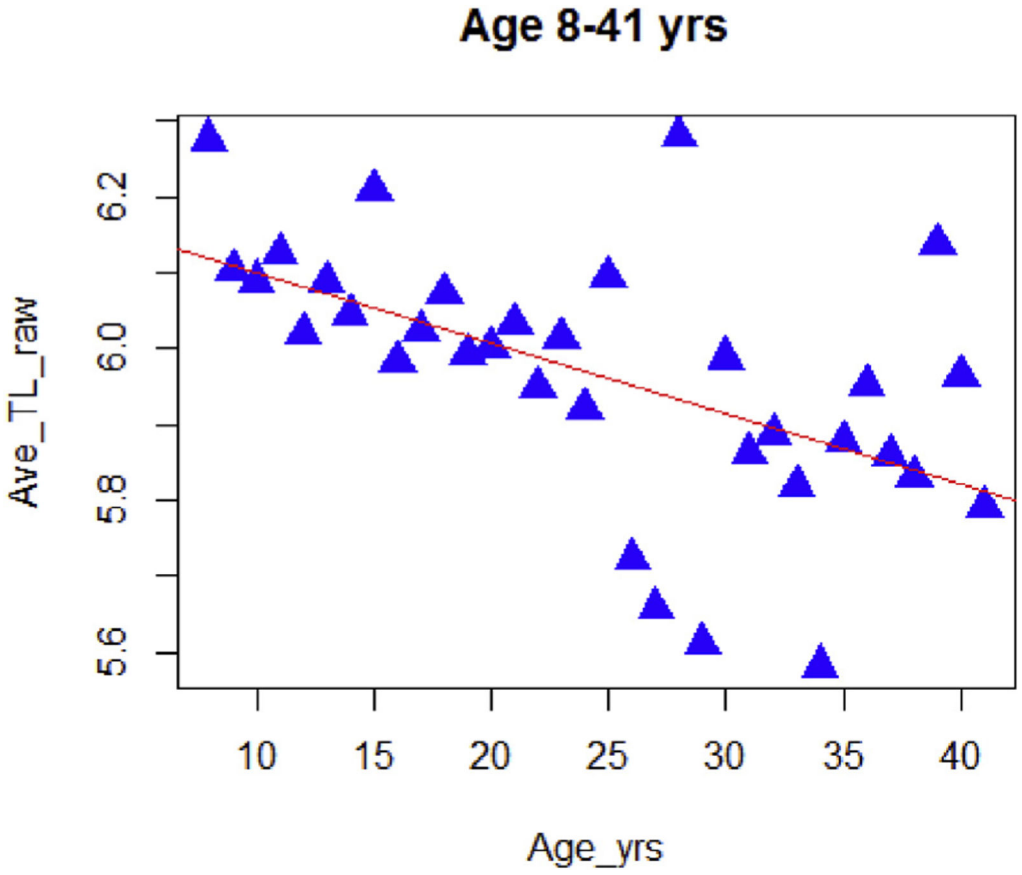

FIG E2.

Relationship between TL (raw qPCR value) and age (8 to 41 years). The *least squares linear regression line* presents age on the *x-axis* and age group–specific mean TL (raw qPCR values) on the *y-axis*. Older participants from SAGE I were included, which yielded a wider age range.

TABLE I.

Descriptive statistics for SAGE participants (n = 1072)

Characteristic	
Age (y), mean (SD)	14.4 (3.6)
Male sex, no. (%)	535 (50.0)
African ancestry (%), mean (SD)	77.6 (12.3)
Asthma cases, no. (%)	664 (62.0)
Current smokers in household, no. (%)	
0	726 (67.7)
1	240 (22.4)
≥2	106 (9.9)
Recruitment season, no. (%)	
Spring	271 (25.3)
Summer	416 (38.8)
Fall	165 (15.4)
Winter	220 (20.5)
Maternal education, no. (%)	
Less than high school	142 (13.2)
High school	293 (27.3)
Greater than high school	637 (59.4)
TL (kb/diploid), mean (SD)	2490.4 (1265.1)
Past year average, mean (SD)	
O_3 (ppb)	21.6 (4.1)
$PM_{2.5}$ ($\mu g/m^3$)	9.3 (1.9)
Steroid medication in asthmatic patients, no. (%)	
ICS use	349 (52.6)
Any steroid use	446 (67.2)

Proportions might not sum to 100% because of round-up error.

TABLE II.

Association between aTL and annual average exposures to O_3 and $PM_{2.5}$: 2006–2014

Variable name (n = 1072)	aTL (kb [95% CI])	*P* value
O_3 (ppb)	−37.1 (−66.7 to −7.4) *	.01
$PM_{2.5}$ (μg/m^3)	−57.1 (−118.1 to 3.9)	.07
African ancestry (%)	8.4 (0.8 to 15.9) *	.03
Age (y)	−26.1 (−52.3 to 0.1)	.05

Estimates are provided as change in mean aTL (kb/diploid) for 1-ppb and 1 μg/m^3 increases in O_3 and $PM_{2.5}$, respectively, for annual average (past-year) exposures.

A multivariable linear regression model was adjusted for air pollutants (O_3 and $PM_{2.5}$), age, sex, African ancestry, asthma status, number of household smokers, recruitment season, and maternal education.

* $P < .05$.

TABLE III.

Association between aTL and annual average exposures to O_3 and $PM_{2.5}$ by asthma status: 2006–2014

	aTL (kb [95% CI])	
Exposure	**Asthmatic patients (n = 664)**	**Nonasthmatic subjects (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) *

Estimates are provided as change in mean aTL (kb/diploid) for 1-ppb and 1 μg/m^3 increases in O_3 and $PM_{2.5}$, respectively, for annual average (past year) exposures.

Multivariable linear regression models were adjusted for air pollutants (O_3 and $PM_{2.5}$), age, sex, African ancestry, number of household smokers, recruitment season, and maternal education.

* $P < .05$.

TABLE IV.

Interaction between exposure to air pollution and steroid use on aTL among the asthma cases

Interaction	No. of asthma cases = 664	aTL (kb [95% CI])	*P* value
O_3 × ICS[†]	n = 349	−6.9 (−59.5 to 45.7)	.79
$PM_{2.5}$ × ICS[†]	n = 349	164.1 (18.6 to 309.6)	.03[*]
O_3 × any steroid[‡]	n = 446	3.9 (−54.1 to 61.9)	.89
$PM_{2.5}$ × any steroid[‡]	n = 446	175.9 (−1.3 to 353.0)	.06

Estimates are provided as change in mean aTL (kb/diploid) for 1-ppb and 1 $\mu g/m^3$ increases in O_3 and $PM_{2.5}$, respectively, for annual average (past-year) exposures.

[*] $P < .05$.

[†] Multivariable linear regression models were adjusted for air pollutants (O_3 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 (O_3 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.

TABLE V.

Association between aTL and annual average exposures to O_3 and $PM_{2.5}$ by steroid use subgroups: 2006–2014

	aTL (kb [95% CI])	
Exposure	**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)	**Nonsteroid (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) *

Estimates are provided as change in mean aTL (kb/diploid) for 1-ppb and 1 μg/m^3 increases in O_3 and $PM_{2.5}$, respectively, for annual average (past-year) exposures.

Multivariable linear regression models were adjusted for air pollutants (O_3 and $PM_{2.5}$), age, sex, African ancestry, number of household smokers, recruitment season, and maternal education.

* $P < .05$.

TABLE E1.

Summary of baseline characteristics of included versus not included SAGE participants

Characteristic	Included (n = 1072)	Not included (n = 638)	*P* value
Age (y), mean (SD)*	14.4 (3.6)	15.2 (4.0)	4.6e-05
No. missing	0	0	
Male, no. (%)†	535 (50.0)	284 (44.5)	.03
No. missing	0	0	
African ancestry (%), mean (SD)*	77.6 (12.3)	79.0 (11.3)	.03
No. missing	0	70	
Asthma cases, no. (%)†	664 (62.0)	280 (43.9)	5.6e-13
No. missing	0	0	
Current smokers in household, no. (%)†			
0	726 (67.7)	171 (75.3)	.03
1	240 (22.4)	43 (18.9)	.3
≥2	106 (9.9)	13 (5.7)	.06
No. missing	0	411	
Recruitment season, no. (%)†			
Spring	271 (25.3)	44 (21.0)	.2
Summer	416 (38.8)	83 (39.5)	.9
Fall	165 (15.4)	35 (16.7)	.7
Winter	220 (20.5)	48 (22.9)	.5
No. missing	0	428	
Maternal education, no. (%)†			
Less than high school	142 (13.2)	117 (19.4)	.001
High school	293 (27.3)	170 (28.2)	.7
Greater than high school	637 (59.4)	316 (52.4)	.006
No. missing	0	35	

Proportions might not sum to 100% because of round-up error.

*The unpaired 2-sample *t* test was performed for continuous variables.

†The 2-proportions *z* test was applied for categoric variables.

TABLE E2.

aTLs and air pollutant levels by recruitment season

	Spring (n = 271)	Summer (n = 416)	Fall (n = 165)	Winter (n = 220)
aTL (kb)	2454.8 ± 1223.7	2498.6 ± 1229.8	2592.7 ± 1489.3	2442.1 ± 1201.1
Age (y)	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

All values are reported as means ± SDs.

TABLE E3.

Interaction between exposure to air pollution and asthma status on aTLs

Annual average (n = 1072)	aTL (kb [95% CI])	*P* value
O_3 × asthma status	32.8 (−18.4 to 84.1)	.21
$PM_{2.5}$ × asthma status	36.9 (−79.3 to 153.1)	.53

Estimates are provided as change in mean aTL (kb/diploid) for 1-ppb and 1 $\mu g/m^3$ increases in O_3 and $PM_{2.5}$, respectively, for annual average (past-year) exposures.

Multivariable linear regression models were adjusted for air pollutants (O_3 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.

TABLE E4.

Association between aTLs and annual average exposures to O_3 and $PM_{2.5}$ among asthmatic cases: 2006–2014

	aTL (kb [95% CI), n = 645	
Exposure	**Base models unadjusted for duration of asthma**	**Base models adjusted 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 were adjusted for air pollutants (O_3 and $PM_{2.5}$), age, sex, African ancestry, number of household smokers, recruitment season, and maternal education.

TABLE E5.

Associations between covariates and aTL in SAGE participants

Variable (n = 1072)	aTL (kb [95% CI])†	aTL (kb [95% CI])‡
Age	−15.8 (−32.5 to 0.9)	−26.1 (−52.3 to 0.1)
Sex		
Female (referent)	1.0	1.0
Male	−52.2 (−170.8 to 66.3)	−71.1 (−257.3 to 115.1)
African ancestry proportion	5.9 (1.1 to 10.7) *	8.4 (0.8 to 15.9) *
Asthma status		
Control (referent)	1.0	1.0
Case	80.7 (−46.3 to 207.8)	121.2 (−78.5 to 320.9)
Smoking		
None (referent)	1.0	1.0
1	29.4 (−114.4 to 173.2)	25.3 (−201.0 to 251.6)
≥2	160.0 (−41.6 to 361.6)	229.2 (−87.6 to 546.0)
Recruitment season		
Fall (referent)	1.0	1.0
Spring	−61.6 (−251.5 to 128.3)	−133.6 (−437.5 to 170.3)
Summer	−2.1 (−178.7 to 174.5)	27.4 (−250.8 to 305.6)
Winter	−77.7 (−276.5 to 121.1)	−164.3 (−503.8 to 175.2)
Maternal education		
Greater than high school (referent)	1.0	1.0
Less than high school	9.0 (−169.8 to 187.9)	−43.9 (−328.5 to 240.7)
High school	−29.9 (−166.4 to 106.6)	−67.4 (−282.3 to 147.5)

*P < .05.

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

‡Regression model was adjusted for annual average (past-year) exposure to O_3 and $PM_{2.5}$, age, sex, African ancestry (as a percentage), asthma status, number of household smokers, recruitment season, and maternal education.

TABLE E6.

Univariate analysis between age and age group–specific mean TL (raw qPCR values)

Fig E1 (8–21 y)			Fig E2 (8–41 y)*		
	Estimate	***P* Value**		**Estimate**	***P* Value**
Age	−0.012	.01	Age	−0.009	.0008

* In Fig E2 we included older participants from SAGE I, which yielded a wider age range (8–41 years).

TABLE E7.

Oligomers used for aTL assay

	Oligomer name	Oligomer sequence (5'−3')
Standards	Telomere	(TTAGGG)14
	36B4	CAGCAAGTGGGAAGGTGTAATCCGTCTCCACAGACAAGGCCAGGACTCGTTTG
PCR primers	teloF	CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGG TTTGGGTT
	teloR	GGCTTGCCTTACCCTTACCCTTACCC TTACCCTTACCCT
	*36B4*F	CAGCAAGTGGGAAGGTGTAATCC
	36B4R	CCCATTCTATCATCAACGGGTACAA