HHS Public Access

Author manuscript

J Occup Environ Med. Author manuscript; available in PMC 2018 May 01.

Published in final edited form as:

J Occup Environ Med. 2017 May ; 59(5): 446–452. doi:10.1097/JOM.0000000000000996.

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

Eunice Y. Lee, MS[1,*], **Jue Lin, PhD**[3], **Elizabeth M. Noth, PhD**[1], **S. Katharine Hammond, PhD**[1], **Kari C. Nadeau, MD, PhD**[2], **Ellen A. Eisen, ScD**[1], and **John R. Balmes, MD**[1,4]

[1]Division of Environmental Health Sciences, School of Public Health, University of California, Berkeley, Berkeley, California, United States of America

[2]Division of Immunology and Allergy, School of Medicine, Stanford University, Stanford, California, United States of America

[3]Department of Biochemistry and Biophysics, University of California, San Francisco, San Francisco, California, United States of America

[4]Division of Occupational and Environmental Medicine, School of Medicine, University of California, San Francisco, San Francisco, California, United States of America

Abstract

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

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

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

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

[*]Corresponding author: John R. Balmes, Division of Environmental Health Sciences, School of Public Health, University of California, 50 University Hall, Berkeley, CA 94720, USA, Telephone: 1- 415-206-8953, Fax: 1-510-642-5815, john.balmes@ucsf.edu.

Conflict of interest (COI): None declared

Author Contributions

Conceived the study, analyzed the data, and was the primary author of the paper: EL. Provided intellectual input and contributed to the writing of the paper: JB, EE, KH. Telomere length measurement and interpreting results: JL. Designed the study and provided PBMC samples: KN, MP. Provided PAH exposure data, edited and reviewed the paper: KH, BN. The authors declare that they have no conflicting interest.

Introduction

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

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

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

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

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

chemical compounds characterized by fused benzene rings (20). PAHs are produced during incomplete combustion of organic matter. They exist in ambient air in both gas and particle phases (adsorbed to particulate matter). In many urban environments, motor vehicle exhaust is the main source of high-molecular-weight PAHs (four to six rings), which are more carcinogenic and mutagenic than low-molecular-weight PAHs (two- and three-rings) (21). Ambient concentrations of PAHs in the United States range from 0.02–1.2 ng/m^3 in rural areas, and 0.15–57.1 ng/m^3 in urban environments (22–25). PAHs are ubiquitous ambient air pollutants in Fresno and can be transformed into quinones in the atmosphere (25–27). Quinones can serve as catalysts in redox cycling and generate free radicals (26,27).

Methods

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

Study subjects

Subjects were selected from a larger population of children enrolled in an ongoing study of asthma in Fresno, CA (Figure 1). They were age 11 to 18 years old, living in Fresno, California. Fresno is located in the center of the San Joaquin Valley, which is part of the Central Valley in California. Fresno is the second-most polluted city in the United States, in terms of 24-hour average $PM_{2.5}$ (28) and has a high prevalence of asthma (29). For the pilot study, 14 subjects were selected from high- and low-exposure groups, as defined by annual average 24-hour outdoor residential exposure to PAHs in the 12 months prior to their blood draw (2009–2012). The high-exposure group was defined as above the 80^{th} percentile of PAH exposures and the low-exposure group below the 10^{th} percentile. An equal number of subjects (n=7) were selected from the high- and low-exposure groups.

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

Individual PAH exposure estimates

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

residential samples from a subset of FACES participants' homes. .The filter-based PAH samples provided concentrations for 14 PAHs. However, we chose to use the sum of the mass concentrations of PAHs with 4-, 5- or 6-rings in this analysis as a metric representing the less volatile, particle-bound PAHs. This selected group of PAHs (PAH456) had a good correlation with the continuous measure of PAHs we were using in the spatial-temporal model. Outdoor, residential 24-hour PAH456 concentrations were used as the dependent variable in a mixed-effects regression model with a large number of independent land use and meteorological variables. Good agreement between predicted and measured concentrations of PAH456 was reported with the final model. The model parameters were used to calculate individual daily exposure to outdoor residential PAH456. More information on the model selection/parameters and field sampling of PAH456 can be found in Noth et al., 2011 (27).

Telomere length measurement

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

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

The primers for the telomere PCR were *tel1b* [5′-CGGTTT$(GTTTGG)_5$GTT-3′], used at a final concentration of 100 nM, and *tel2b* [5′-GGCTTG$(CCTTAC)_5$CCT-3′], used at a final concentration of 900 nM. The primers for the single-copy gene (human beta-globin) PCR were *hbg1* [5′ GCTTCTGACACAACTGTGTTCACTAGC-3′], used at a final concentration of 300 nM, and *hbg2* [5′-CACCAACTTCATCCACGTTCACC-3′], used at a final concentration of 700 nM. The final reaction mix contained 20 mM Tris-HCl, pH 8.4; 50 mM KCl; 200 μM each dNTP; 1% DMSO; 0.4× Syber Green I; 22 ng E. coli DNA per reaction; 0.4 Units of Platinum Taq DNA polymerase (Invitrogen Inc.) per 11 microliter reaction; 0.5 – 10 ng of genomic DNA. Tubes containing 26, 8.75, 2.9, 0.97, 0.324 and 0.108ng of a reference DNA (from Hela cancer cells) were included in each PCR run so that the quantity of targeted templates in each research sample can be determined relative to the reference DNA sample by the standard curve method. The same reference DNA was used for all PCR runs.

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

more than 7%, the sample was run a third time and the two closest values were reported. The coefficient of variation (CV) for this study was typically 2.5%.

Statistical analysis

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

Results

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

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

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

Discussion

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

Air Pollution and Telomere Length

The relationship between PAH exposure and telomere length we observed in this study of adolescents is consistent with studies in healthy adults that have shown telomere shortening with increasing air pollution levels (13,14,36–38). For example, Hoxha et al. reported mean leukocyte telomere length (LTL) among traffic officers in Milan, Italy was 1.10 (95% CI: 1.04–1.16) compared to a mean LTL in office workers of 1.27 (95% CI: 1.20–1.35) (14). In our younger participants, the mean telomere length of the subjects with the lowest PAH exposure was 1.38, whereas the telomere length of the participant with the highest PAH exposure was 0.96.

Previous studies have reported a dose-response relationship between PAH exposure and biomarkers of oxidative stress (39,40). Although preliminary pilot data, our results are consistent with the hypothesis that exposure to ambient PAHs (largely generated during combustion of diesel and gasoline fuels in Fresno) leads to oxidative stress, which in turn causes telomere shortening.

Age and Telomere Length

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

Sex and Telomere Length

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

Asthma and Telomere Length

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

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

Strengths and Limitations

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

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

Conclusions

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

Acknowledgments

We acknowledge the help of field officers, researchers and scientists who were involved with the study- Ms. Leah Melendez, Tim Tyner, Michael Peterson, Cynthia Appel, and Dr. Ira Tager. We thank the subjects, their families, and the community of Fresno.

Funding sources: Funding for this research was provided by the Global Health Research Foundation and a NIEHS grant (R01 HL081521). This work was also performed as part of the University of California, Berkeley/Stanford Children's Environmental Health Center, pre-Center funded (2010 – 2014) by NIEHS 1P20ES018173 and EPA RD-83459601. This publication was made possible by US EPA grant RD-83459601. Its contents are solely the responsibility of the grantee and do not necessarily represent the official views of the US EPA. Further, the US EPA does not endorse the purchase of any commercial products or services mentioned in the publication.

References

1. Who. WHO's Ambient Air Pollution database; Update 2014 Data summary of the AAP database. 2014. p. 2-7.Available from: http://www.who.int/phe/health_topics/outdoorair/databases/cities/en/
2. Jerrett M. Atmospheric science: The death toll from air-pollution sources. Nature. 2015; 525(7569): 330–1. [PubMed: 26381981]
3. Dockery D. Acute Respiratory Effects of Particulate Air Pollution. Annual Review of Public Health. 1994 Jan; 15(1):107–32.
4. Brook RD, Franklin B, Cascio W, Hong Y, Howard G, Lipsett M, et al. Air pollution and cardiovascular disease: A statement for healthcare professionals from the expert panel on population

and prevention science of the American Heart Association. Circulation. 2004:2655–71. [PubMed: 15173049]

5. Dockery DW, Pope CA, Xu X, Spengler JD, Ware JH, Fay ME, et al. An Association between Air Pollution and Mortality in Six U.S. Cities. N Engl J Med. 1993; 329(24):1753–9. [PubMed: 8179653]
6. Health Effects Institute. Traffic-related air pollution: a critical review of the literature on emissions, exposure, and health effects. Health Effects Institute; 2010.
7. Pope CA. Cardiovascular Mortality and Long-Term Exposure to Particulate Air Pollution: Epidemiological Evidence of General Pathophysiological Pathways of Disease. Circulation. 2003; 109(1):71–7. [PubMed: 14676145]
8. Pope CA, Burnett RT, Thun MJ, et al. Lung Cancer, Cardiopulmonary Mortality, and Long-term Exposure to Fine Particulate Air Pollution. JAMA : the journal of the American Medical Association. 2002; 287(9):1132–1141. [PubMed: 11879110]
9. Stanek LW, Brown JS, Stanek J, Gift J, Costa DL. Air pollution toxicology-a brief review of the role of the science in shaping the current understanding of air pollution health risks. Toxicological Sciences. 2011
10. Lodovici M, Bigagli E. Oxidative stress and air pollution exposure. Journal of Toxicology. 2011
11. Risom L, Møller P, Loft S. Oxidative stress-induced DNA damage by particulate air pollution. Mutat Res. 2005; 592(1–2):119–37. [PubMed: 16085126]
12. Valavanidis A, Vlachogianni T, Fiotakis K, Loridas S. Pulmonary oxidative stress, inflammation and cancer: Respirable particulate matter, fibrous dusts and ozone as major causes of lung carcinogenesis through reactive oxygen species mechanisms. Int J Environ Res Public Health. 2013; 10(9):3886–907. [PubMed: 23985773]
13. 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]
14. 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]
15. Blackburn EH. Structure and function of telomeres. Nature. 1991; 350(6319):569–73. [PubMed: 1708110]
16. Blackburn EH. Telomere states and cell fates. Nature. 2000; 408(6808):53–6. [PubMed: 11081503]
17. Von Zglinicki T. Oxidative stress shortens telomeres. Trends in Biochemical Sciences. 2002:339–44. [PubMed: 12114022]
18. Saretzki G. Telomeres, Senescence and Longevity: The Role of Oxidative Stress and Antioxidants. Curr Pharmacogenomics. 2005; 3(2):129–56.
19. Frenck RW Jr, Blackburn EH, Shannon KM, Frenck RW, Blackburn EH, Shannon KM. The rate of telomere sequence loss in human leukocytes varies with age. Proc Natl Acad Sci U S A. 1998; 95(10):5607–10. [PubMed: 9576930]
20. Boström C-E, Gerde P, Hanberg A, et al. Cancer risk assessment, indicators, and guidelines for polycyclic aromatic hydrocarbons in the ambient air. Environmental Health Perspectives. 2002; 110(Suppl 3):451–488. [PubMed: 12060843]
21. Stogiannidis E, Laane R. Source Characterization of Polycyclic Aromatic Hydrocarbons by Using Their Molecular Indices: An Overview of Possibilities. Reviews of Environmental Contamination and Toxicology. 2014:49–133.
22. Jung KH, Patel MM, Moors K, et al. Effects of Heating Season on Residential Indoor and Outdoor Polycyclic Aromatic Hydrocarbons, Black Carbon, and Particulate Matter in an Urban Birth Cohort. Atmospheric environment. 2010; 44(36):4545–4552. [PubMed: 20938487]
23. Mishra N, Ayoko GA, Morawska L. Atmospheric polycyclic aromatic hydrocarbons in the urban environment: Occurrence, toxicity and source apportionment. Environmental Pollution. 2016; 208:110–7. [PubMed: 26428471]
24. Noth EM, Lurmann F, Northcross A, Perrino C, Vaughn D, Hammond SK. Spatial and temporal distribution of polycyclic aromatic hydrocarbons and elemental carbon in Bakersfield, California. Air Quality, Atmosphere & Health. 2016 Sep; 9(8):899–908.

25. Noth EM, Hammond SK, Biging GS, Tager IB. A spatial-temporal regression model to predict daily outdoor residential PAH concentrations in an epidemiologic study in Fresno, CA. Atmos Environ. 2011; 45(14):2394–403.

26. Chung MY, Lazaro RA, Lim D, Jackson J, Lyon J, Rendulic D, et al. Aerosol-Borne Quinones and Reactive Oxygen Species Generation by Particulate Matter Extracts. Environmental Science & Technology. 2006; 40(16):4880–6. [PubMed: 16955881]

27. Kelly FJ. Oxidative stress: its role in air pollution and adverse health effects. Occup Environ Med [Internet]. 2003; 60(8):612–6. Available from: http://oem.bmj.com/cgi/doi/10.1136/oem.60.8.612.

28. US EPA O of A. The Green Book Nonattainment Areas | Green Book | US EPA. [cited 2016 Mar 7]; Available from: https://www3.epa.gov/airquality/greenbook/

29. Fresno - California Breathing. [cited 2016 Mar 7]. Available from: http://californiabreathing.org/asthma-data/county-asthma-profiles/fresno-county-asthma-profile

30. Nadeau K, McDonald-Hyman C, Noth EM, Pratt B, Hammond SK, Balmes J, et al. Ambient air pollution impairs regulatory T-cell function in asthma. J Allergy Clin Immunol. 2010; 126(4):845–52. e10. [PubMed: 20920773]

31. Hew KM, Walker AI, Kohli A, Garcia M, Syed A, Mcdonald-Hyman C, et al. Childhood exposure to ambient polycyclic aromatic hydrocarbons is linked to epigenetic modifications and impaired systemic immunity in T cells. Clin Exp Allergy. 2015; 45(1):238–48. [PubMed: 25048800]

32. Mann JK, Balmes JR, Bruckner TA, Mortimer KM, Margolis HG, Pratt B, et al. Short-term effects of air pollution on wheeze in asthmatic children in Fresno, California. Environ Health Perspect. 2010; 118(10):1497–502. [PubMed: 20570778]

33. Gale SL, Noth EM, Mann J, Balmes J, Hammond SK, Tager IB. Polycyclic aromatic hydrocarbon exposure and wheeze in a cohort of children with asthma in Fresno, CA. J Expo Sci Environ Epidemiol. 2012; 22(September 2011):386–92. [PubMed: 22549720]

34. Cawthon RM. Telomere measurement by quantitative PCR. Nucleic Acids Res [Internet]. 2002; 30(10):e47. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19129229.

35. Lin J, Epel E, Cheon J, Kroenke C, Sinclair E, Bigos M, et al. Analyses and comparisons of telomerase activity and telomere length in human T and B cells: Insights for epidemiology of telomere maintenance. J Immunol Methods. 2010; 352(1–2):71–80. [PubMed: 19837074]

36. Weng N. Interplay between telomere length and telomerase in human leukocyte differentiation and aging. J Leukoc Biol. 2001; 70(6):861–7. [PubMed: 11739547]

37. Jiang H, Ju Z, Rudolph KL. Telomere shortening and ageing. Zeitschrift fur Gerontologie und Geriatrie. 2007:314–24. [PubMed: 17943234]

38. Shammas MA. Telomeres, lifestyle, cancer, and aging. Curr Opin Clin Nutr Metab Care. 2011; 14(1):28–34. [PubMed: 21102320]

39. Jeng HA, Pan C-H, Diawara N, Chang-Chien G-P, Lin W-Y, Huang C-T, et al. Polycyclic aromatic hydrocarbon-induced oxidative stress and lipid peroxidation in relation to immunological alteration. Occup Environ Med. 2011; 68(9):653–8. [PubMed: 21126960]

40. Frenkel K, Wei L, Wei H, Karkoszka J. Polycyclic Aromatic Hydrocarbons (PAH) Induce Oxidative Stress and Oxidative DNA Modification - Characteristics of Tumor Promotion. Polycycl Aromat Compd. 1994; 6(1–4):151–60.

41. Hodes RJ, Hathcock KS, Weng N. Telomeres in T and B cells. Nat Rev Immunol. 2002; 2(9):699–706. [PubMed: 12209138]

42. 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(2):215–20. [PubMed: 20855079]

43. Gardner M, Bann D, Wiley L, Cooper R, Hardy R, Nitsch D, et al. Gender and telomere length: Systematic review and meta-analysis. Exp Gerontol. 2014; 51(1):15–27. [PubMed: 24365661]

44. Houben JMJ, Moonen HJJ, van Schooten FJ, Hageman GJ. Telomere length assessment: Biomarker of chronic oxidative stress? Free Radical Biology and Medicine. 2008:235–46. [PubMed: 18021748]

45. Moore WC, Bleecker ER, Curran-Everett D, Erzurum SC, Ameredes BT, Bacharier L, et al. Characterization of the severe asthma phenotype by the National Heart, Lung, and Blood

Institute's Severe Asthma Research Program. J Allergy Clin Immunol. 2007; 119(2):405–13. [PubMed: 17291857]

46. Murdoch JR, Lloyd CM. Chronic inflammation and asthma. Mutat Res. 2010; 690(1–2):24–39. [PubMed: 19769993]
47. Chen E, Miller GE. Stress and inflammation in exacerbations of asthma. Brain, Behavior, and Immunity. 2007:993–9.
48. Guarnieri M, Balmes JR. Outdoor air pollution and asthma. Lancet. 2014; 383(9928):1581–92. [PubMed: 24792855]
49. Alexis NE, Carlsten C. Interplay of air pollution and asthma immunopathogenesis: A focused review of diesel exhaust and ozone. International Immunopharmacology. 2014:347–55.
50. Klumper C, Kramer U, Lehmann I, von Berg A, Berdel D, Herberth G, et al. Air pollution and cytokine responsiveness in asthmatic and non-asthmatic children. Environ Res. 2015; 138:381–90. [PubMed: 25769127]
51. Mitchell C, Hobcraft J, McLanahan SS, Siegel SR, Berg A, Brooks-Gunn J, et al. Social disadvantage, genetic sensitivity, and children's telomere length. Proc Natl Acad Sci U S A. 2014; 111(16):5944–9. [PubMed: 24711381]

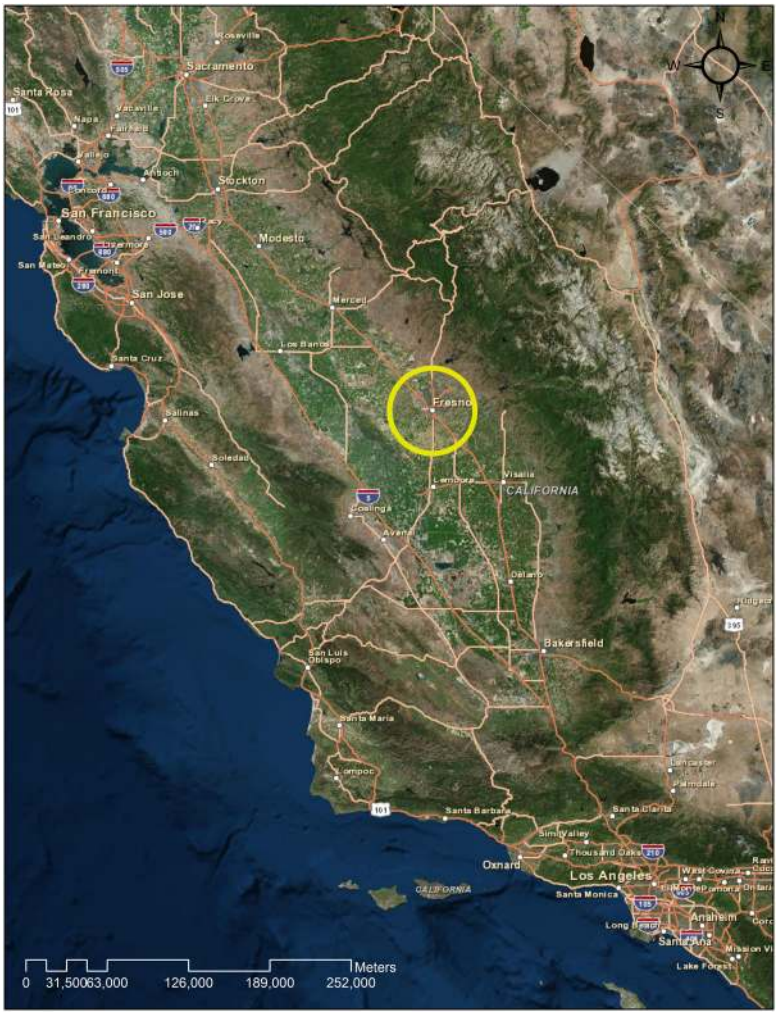

Figure 1.
Location of the study area.

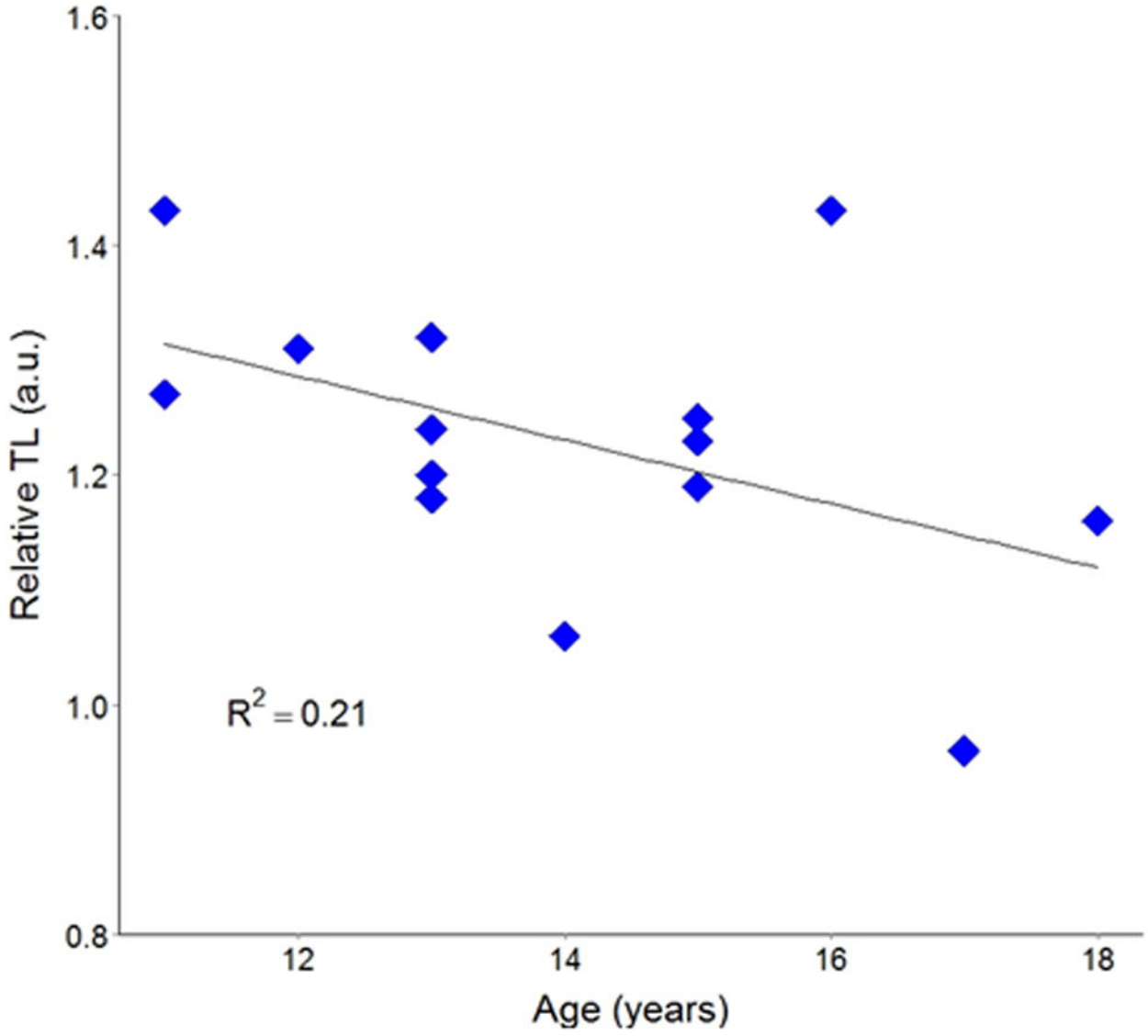

Figure 2.
Scatter plot for age and telomere length.

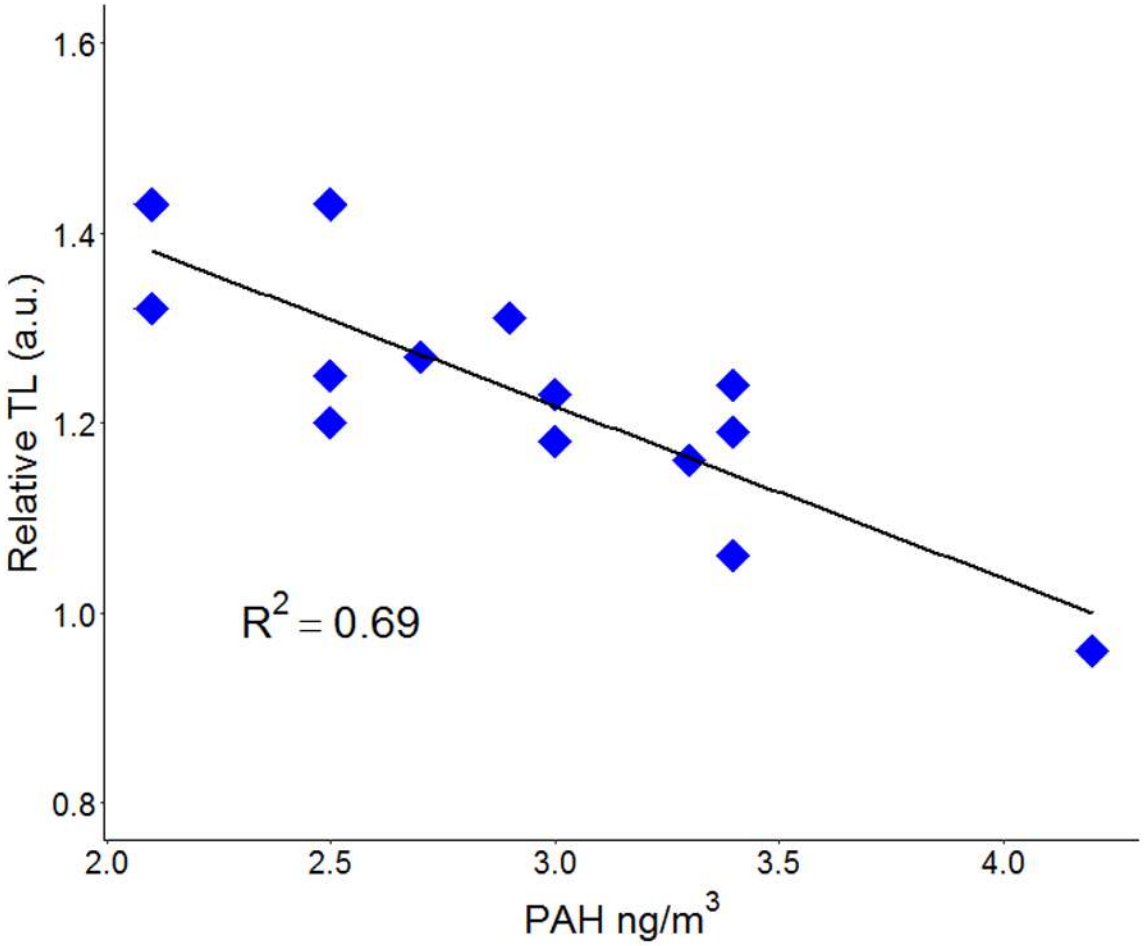

Figure 3.
Scatter plot for PAH exposure and telomere length.

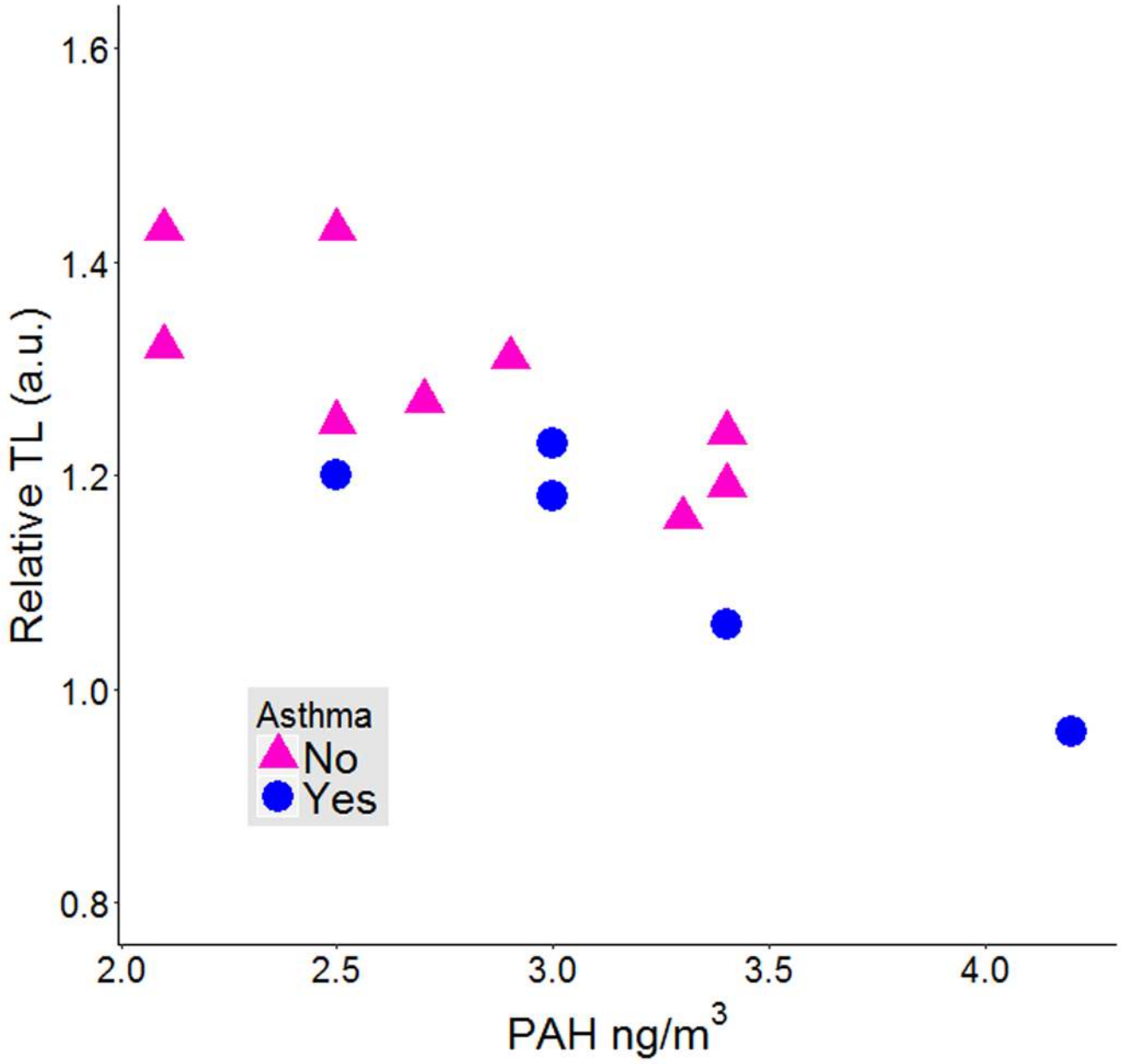

Figure 4.
Scatter plot for telomere length and PAH exposure by asthma status.

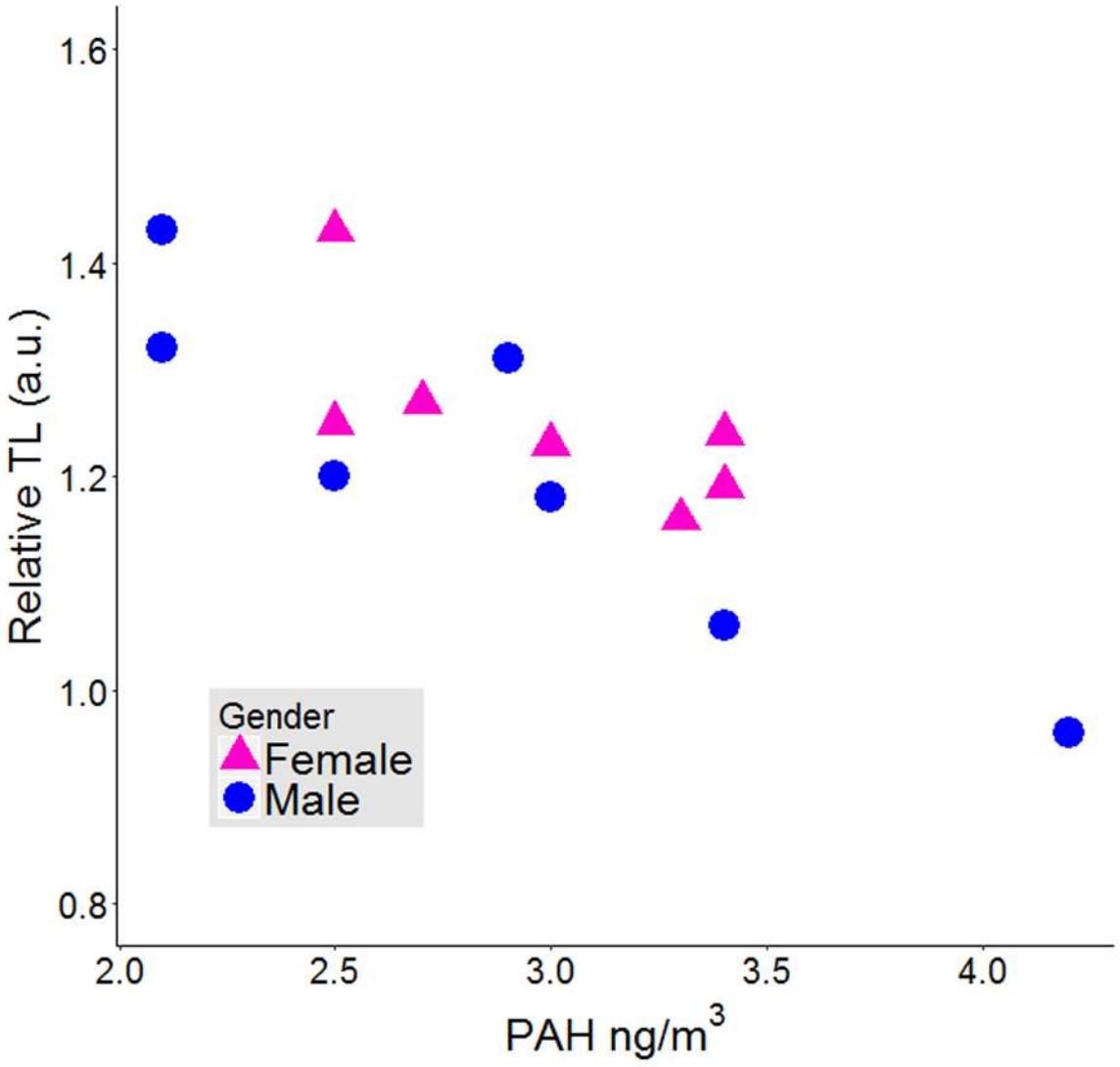

Figure 5.
Scatter plot for telomere length and PAH exposure by gender.

Table 1

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

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

Table 2

Mean age, telomere length and PAHs exposure by subgroups

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

Table 3

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

Predictor	Coef	St.Error	t-value	Pr(>t)
(Intercept)	1.80	0.15	12.00	<.01
PAH (ng/m3)	−0.14	0.04	−3.50	0.01
Age (yr)	−0.0086	0.013	−0.66	0.54
Gender (ref group: male)	−0.04	0.05	−0.80	0.46
Race/ethnicity (ref group: white)	0.01	0.05	0.20	0.79
Asthma status (ref group: asthmatic)	−0.07	0.05	−1.40	0.19

Residual standard error: 0.066 on 8 degrees of freedom

Multiple R-squared: 0.83, Adjusted R-squared: 0.72

F-statistic: 7.849 on 5 and 8 DF, p-value: 0.0059