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Air pollution, ethnicity and telomere length in east London schoolchildren: an observational study

Robert T Walton[1]†, Ian S Mudway[2], Isobel Dundas[3], Nadine Marlin[1], Lee C Koh[3], Layla Aitlhadj[2], Tom Vulliamy[3], Jeenath B. Jamaludin[2], Helen E. Wood[2], Ben M. Barratt[2], Sean Beevers[2], David Dajnak[2], Aziz Sheikh[4], Frank J Kelly[2], Chris J Griffiths[1], Jonathan Grigg[3]

[1] Asthma UK Centre for Applied Asthma Research, Centre for Primary Care and Public Health, Blizard Institute, Queen Mary University of London, London, United Kingdom

[2] MRC-PHE Centre for Environment and Health and NIHR HPRU in Health Impact of Environmental Hazards, King's College London, London, United Kingdom

[3] Blizard Institute, Barts and the London School of Medicine and Dentistry, Queen Mary University of London, London, United Kingdom

[4] Usher Institute of Population Health Sciences and Informatics, The University of Edinburgh, Medical School Doorway 3, Teviot Place, Edinburgh, United Kingdom

†To whom correspondence should be addressed at Centre for Primary Care and Public Health, Blizard Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, 58 Turner St, London E1 2AB, UK

Email r.walton@qmul.ac.uk, Tel: +44 (0)20 7882 2502, Fax: +44 (0)20 7882 2552

PA Mrs Ezinne Chukuka e.chukuka@qmul.ac.uk +44 (0)20 7882 2498

Short running title: London air pollution and telomeres in children

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Keywords

Telomeres, air pollution, ethnicity, lung function, particulate matter, nitrogen oxides.

Abstract

Background: Short telomeres are associated with chronic disease and early mortality. Recent studies in adults suggest an association between telomere length and exposure to particulate matter, and that ethnicity may modify the relationship. However associations in children are unknown.

Objectives: We examined associations between air pollution and telomere length in an ethnically diverse group of children exposed to high levels of traffic derived pollutants, particularly diesel exhaust, and to environmental tobacco smoke.

Methods: Oral DNA from 333 children (8-9 years) participating in a study on air quality and respiratory health in 23 inner city London schools was analysed for relative telomere length using monochrome multiplex qPCR. Annual, weekly and daily exposures to nitrogen oxides and particulate matter were obtained from urban dispersion models (2008-10) and tobacco smoke by urinary cotinine. Ethnicity was assessed by self-report and continental ancestry by analysis of 28 random genomic markers. We used linear mixed effects models to examine associations with telomere length.

Results: Telomere length increased with increasing annual exposure to NO_x (model coefficient 0.003, [0.001, 0.005], $p<0.001$), NO_2 (0.009 [0.004, 0.015], $p<0.001$), $PM_{2.5}$ (0.041, [0.020, 0.063], $p<0.001$) and PM_{10} (0.096, [0.044, 0.149], $p<0.001$). There was no association with environmental tobacco smoke. Telomere length was increased in children reporting black ethnicity (22% [95% CI 10%, 36%], $p<0.001$)

Conclusions: Pollution exposure is associated with longer telomeres in children and genetic ancestry is an important determinant of telomere length. Further studies should investigate

both short and long-term associations between pollutant exposure and telomeres in childhood and assess underlying mechanisms.

Introduction

Short telomere length in circulating leucocytes is associated with common diseases that cause substantial mortality and morbidity across human populations (Calado and Young 2009). Environmental factors, particularly those inducing cellular oxidative stress, are thought to be important in determining the rate of telomere erosion (von Zglinicki et al. 2005). It has been suggested that exposure to air pollution causes oxidative stress (Miller 2014) and that vehicle emissions contribute significantly to the oxidative burden (De Prins et al. 2014; Rosa et al. 2014). Particulate matter collected from roadside locations in London, has remarkably high oxidative potential with significant contributions both from vehicle exhausts and mechanical abrasion of brakes and tyres (Kelly et al. 2011). Studies in adults have shown associations between short telomere length and traffic-related pollution: black carbon (McCracken et al. 2010; Pieters et al. 2015); aromatic hydrocarbons (Hoxha et al. 2009) although in one study the direction of the association was contradictory (Hou et al. 2012)

The long-term consequences of shortened telomeres on health are substantial (Grahame and Schlesinger 2012). There are strong associations with coronary heart disease (Brouilette et al. 2007; Codd et al. 2013) and studies in other cohorts show associations with all-cause mortality, which persist when estimates are adjusted for heart disease risk (Fitzpatrick et al. 2011), although these findings are not universal (Svensson et al. 2014). Meta analyses show that short telomeres in adults are associated with common solid tumours particularly bladder, oesophageal, gastric and renal (Wentzensen et al. 2011). Whilst shared environmental factors

and reverse causality might explain some of these associations, one recent large study in adults found a strong relationship between germline genetic determinants of telomere length and cancer risk which suggests a direct causal link (Iles et al. 2014).

The rate of telomere loss is greatest in young children (Aubert and Lansdorp 2008) and the decline in length then continues at a slower rate throughout adulthood (Yamaguchi et al. 2005). Thus telomere loss in childhood is a potentially important factor governing ultimate telomere length in adults. The effects of environmental factors might be expected to be greatest in childhood when most telomere attrition is occurring. However whilst there is some evidence that prenatal exposure to tobacco smoke has a lasting effect on telomere length (Theall et al. 2013), there are to date no studies examining associations between exposure to particulate matter and telomere length in children.

There is some preliminary evidence that telomere length in adults is related to continental ancestry, such that Africans have longer telomeres than Europeans (Needham et al. 2013). However ethnicity has not been considered in previous reports of environmental effects on telomeres arising from exposure to pollutants.

Based in an overview of the adult data, we hypothesised that telomere length in children would be inversely related to pollution exposure. Thus we examined associations between air pollution and telomere length in children from African, Asian and European ethnic backgrounds in an area of east London with high traffic density and a high proportion of diesel vehicles.

Methods

Study Design and Setting : Children aged 8-9 years in 23 schools in east London (Tower Hamlets and Hackney) participated in the EXHALE (Exploration of Health and Lungs in the Environment) study examining the impact of air pollution on respiratory health (Wood et al. 2015). Participating schools were selected to achieve a high contrast in urban pollutant exposure based on urban dispersion models at 20x20m resolution (London 2008). All children gave information on respiratory health using a standard questionnaire (ISAAC 1998) together with saliva and urine samples in a sequential cross-sectional study over three consecutive winters (Nov-Mar, 2008-11). Parents gave written consent and children verbal assent. The study was approved under research ethics and governance frameworks (Ref 08-H0704-139). The first year of the study was an internal pilot where study procedures for obtaining measurements and biological samples in schools were optimised.

Participants: All children on school registers with parental consent were eligible and were sent questionnaires. Assessments were conducted one day at each school, each year and those children not present were not followed up. Demographic data including ethnicity were obtained from school records. Deprivation score for each child was assigned by home postcode (http://dclgapps.communities.gov.uk/imd/imd-by-postcode.html). Height was measured using a portable stadiometer and recorded to 0.1 cm by trained investigators. Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared. Level of obesity was classified using International Obesity Task Force criteria (Cole et al. 2000).

Air pollution: Annual exposures to NO_x, NO_2, PM_{10} and $PM_{2.5}$ were estimated using Kings College London, UK urban models (2008-2010) (Beevers et al. 2013), with residential and school address coordinates and assuming 15.6% time at school (7 hour school day, for 5 days per week, 39 weeks per year). Annual exposures were calculated as a calendar average for each year. Acute exposure estimates were derived at the address point by scaling annual mean concentrations according to a 'Nowcast' factor calculated for each pollutant for periods immediately prior to evaluation of lung function. The Nowcast factor is the ratio between concentrations measured by a local subset of continuous air pollution monitoring sites in the prior period, and the annual mean of measurements at the same sites. For this study 'Nowcast' scaling factors were calculated for the 24 hours and seven days before the school visits, working backwards from 10 am on the visit day to reflect both acute and sub-chronic exposure periods. To derive NOx and NO_2, scaling factors measurements were averaged across 14-17 urban background and roadside sites within and surrounding the London Boroughs of Tower Hamlets and Hackney, based on data availability. For the PM_{10} and $PM_{2.5}$ scaling factors measurements from 9-13 and 14-20 background and roadside sites were averaged, respectively.

Environmental tobacco smoke: Urinary cotinine was measured by enzyme linked immunosorbant assay (ELISA) (Product number M155B1, Concateno, Abingdon UK) and corrected for creatinine (Product number 500701, Cayman Chemical Company, Ann Arbor, MI, USA). Children with a cotinine:creatinine ratio of ≥30 ng/mg were defined as positive for tobacco smoke exposure (Henderson et al. 1989).

IgA and cortisol: Salivary IgA was measured using a commercially available ELISA (eBioscience Easy Set-Go! ELISA 88-50600). Cortisol was determined by colorimetric competitive enzyme immunoassay (Enzo Life Sciences, ADI-901-071).

DNA and Genotyping: Genomic DNA was isolated from saliva (OrageneDNA kit OG-250, DNA GenotekInc, Canada), quality assessed (Nanodrop ND-1000 Spectrophotometer, Nanodrop Technologies, Wilmington, DE), Quant-iT™ PicoGreen® assay (Invitrogen) and stored at -80 ^{o}C. DNA quality was confirmed by gel electrophoresis. Genotyping for randomly spaced markers was performed on multiple displacement amplified (MDA) DNA (REPLI g Midi Kit. Qiagen 150045) using GoldenGate genotyping assay on the IlluminaBeadXpress platform (Illumina Inc., San Diego, USA) and analyzed for assay quality control and Hardy Weinberg equilibrium with BeadStudio software. Genotyping success was 99%.

Continental ancestry: 27 randomly spaced single nucleotide polymorphisms were typed and population sub groups were assigned using the STRUCTURE algorithm (Pritchard et al. 2000). Markers were selected from the Hapmap data set using random numbers to locate chromosomal position. The closest marker to the position was selected unless this was known to be related to human disease in which case the next closest marker was chosen. Ten thousand iterations were performed with STRUCTURE for burn-in resulting in convergence with accurate allele frequency estimates. The process was repeated assuming between two and seven subpopulations with the best fit obtained assuming three population components. A numerical value representing each of these components was assigned to each child.

Telomere length: Telomere length was measured from oral DNA using a monochrome multiplex quantitative polymerase chain reaction (MMq-PCR) to compare telomere (T) repeat sequence copy number to a single copy gene (beta globin, S) (Cawthon 2009). Three reference DNA samples were included in each run as internal controls. Sample DNAs were assayed in triplicate and analysed against a standard curve, prepared using threefold serial dilutions of genomic DNA, also assayed in triplicate. MMq-PCR was performed using a

LightCycler480 as described previously (Vulliamy et al. 2011). Each reaction of 15ul contained 7.5µl SYBR Green I Master, 0.5µl of deionised water, 0.5µl for each the four primers (telg and telc at 30µM plus hbgu and hbgd at 6µM) and 5µl of DNA at 2ng/µl. A positive and negative control as well as a reference sample was included in each plate. Telomere length was expressed as T/S ratio based on the delta Ct (Ct telomere/Ct single-gene) derived from the standard curve and normalized to the reference sample.

Respiratory function: Spirometry was performed by trained investigators according to AT/S-ERS guidelines (Miller et al. 2005) with post-bronchodilator measurements of forced expiratory volume in 1 second (FEV1) reported after salbutamol 400 µg by large volume spacer. Flow-volume loops were manually inspected by an experienced reviewer (ID) for quality standards (Pellegrino et al. 2005).

Statistical methods: We hypothesised that telomere length would be inversely associated with exposure to outdoor air pollution and environmental tobacco smoke and that level of deprivation and obesity might modify this association.

All analysis was conducted according to a pre specified analysis plan. We used linear mixed models with a random effect for school to examine associations between relative telomere length and children's characteristics (Box1.). Characteristics included gender, reported ethnicity, body mass index, urinary cotinine and deprivation index adjusting for age, Ig A, cortisol and study year (**Model 1**). Variables found not to be associated (body mass index, urinary cotinine and deprivation score) were dropped from subsequent analysis. We investigated whether using genomic markers to determine continental ancestry instead of reported ethnicity was more informative (**Model 2**) and whether children's lung function was linked to relative telomere length (**Model 3**).

To assess associations between individual air pollutant exposures and relative telomere length we used linear mixed models with random effect for school crude **(Models 4-15)** and adjusting for age, sex, reported ethnicity, Ig A, cortisol and study year **(Models 16-27)**.

Measurements of telomere length were strongly positively skewed (Supplementary Figure 1) and therefore a log transformation was applied. The model coefficients in Tables 2 and 3 are ratios of geometric means which can be interpreted as percentage change. Associations between individual air pollutant exposures (Table 3) were presented per 1 unit ($\mu g/m^3$) increase in exposure and for the difference between the interquartile ranges (25^{th} and 75^{th} centile) of exposure.

Results

DNA was successfully extracted from 988 of 1001 saliva samples collected during the first three years of the EXHALE study, and of these 333 samples had sufficient genomic DNA for telomere analysis (Figure 1). There were no telomere assay failures. Characteristics of the children are reported in Table 1. There were no differences in baseline characteristics between those who had sufficient DNA for telomere analysis and those who did not, apart from a slight excess of boys (55% *v* 49%) (Supplementary Table 1). Median coefficient of variation for the telomere and single copy gene determinations was 2.29 (range 0.08, 10.77) and 0.84 (0.02, 4.39) respectively (Supplementary Figure 2). The median T/S ratio was 3.3 (range 1.7 to 9.1) and quartile coefficient of dispersion 45.5%.

Associations with telomere length: Model 1 in Table 2 shows that reported ethnicity is a major determinant of telomere length, with those reporting black ethnicity having higher T/S ratio than white or Asian children. The model coefficient represents a 22% (95%CI, 10%, 36%) increase in T/S ratio in children with black ethnic background compared to Asian. Girls had 8% lower T/S ratio than boys (95%CI, 2%, 14%). Body mass index, environmental tobacco exposure and index of multiple deprivation score were not associated with telomere length and were dropped from further models. **Model 2** includes information on continental ancestry from genomic markers and confirms that children with African ancestry have increased T/S ratio with a 10% increase in proportion of African ancestry resulting in a 1.6% increase in telomere length. **Model 3** shows an inverse relation between telomere length and respiratory function such that children with higher FEV_1 had a lower T/S ratio corresponding to 11% reduction (95%CI, 2% increase, 21% decrease) per litre of FEV_1. Ig A and Cortisol were not associated with relative telomere length but were nevertheless included in models to address possible confounding. There was no difference in exposure to pollution across ethnic groups (Supplementary Figure 3), in particular there was no association between increasing African ancestry and pollution exposure (Supplementary Figure 4).

Table 3 shows associations between telomere length and exposure to pollution. Children exposed to higher levels of nitrogen oxides and particulate matter had a higher T/S ratio than those experiencing lower levels. A 1µg/m³ increase in NOx, NO_2, $PM_{2.5}$ and PM_{10} was associated with an increase in T/S ratio of 0.4%, 1.2%, 11.6% and 4.7% respectively. Comparing 25th to 75th centile for annual exposure to each pollutant: NOx 2% increase in T/S ratio; NO_2 4%; $PM_{2.5}$ 12%; PM_{10} 6%. The magnitudes of the associations were similar for exposures in the week before the assessment, but were absent when exposures in the previous day were considered.

Discussion

Main findings

In contrast to expectation long-term exposure to traffic related pollution is associated with increased telomere length in cells from salivary samples in children. The association is strongest with $PM_{2.5}$ where children in the highest quartile of pollution exposure had a T/S ratio 15% higher than those in the lowest quartile. Whilst exposure to environmental tobacco smoke was highly prevalent in children taking part in this study (18%) this was not associated with telomere length.

In our highly ethnically diverse population, reported ethnicity was positively associated with telomere length, with black children having significantly longer telomeres than those of other ethnicities (22% black v Asian). We confirmed these findings using genomic markers related to continental origin to give a numerical representation of the proportion of ancestry from Africa, Asia and Europe.

Comparisons with other studies

This is the first study to examine a range of different air pollutants including nitrogen oxides and particulate matter ($PM_{2.5}$, PM_{10}) in the context of telomere length and the first to observe associations with telomere length in children. Previous studies have linked exposure to particulate matter with shorter telomeres in elderly men and suggested increased shortening with increasing age (McCracken et al. 2010). In contrast, one previous study in young adults showed an association between longer telomeres and short term exposure to particulate matter and suggested that longer exposures might be associated with shorter telomeres resulting from a balance between acute effects of inflammation and the longer term effects of oxidative stress (Hou 2012). Our results are consistent with these previous studies and

suggest that in children exposed continuously to high levels of pollution the lengthening effects may predominate.

In contrast to a recent large study in an ethnically diverse population of adults (Needham et al. 2013) we found no relation between low socio economic status and reduced telomere length. Many of the children in our study lived in areas of London suffering high levels of deprivation thus if there were an effect of deprivation on telomere length in children we would be likely to have observed it. Other studies in adults generally confirm a positive relationship between telomere length and socio economic status (Robertson et al. 2012; Surtees et al. 2012). One suggested mediator of this relationship is increased levels of stress (Mitchell et al. 2014) - adding salivary cortisol to the models as an approximation of current stress levels did not change the relation between pollution and telomere length in our study. Salivary Ig A was also included as a potential marker of depressed mucosal immunity, but as with cortisol it did not modify the underlying associations.

In children participating in our study telomere length was shorter in girls (8%) which is in contrast to previous studies in adults (De Meyer et al. 2007; Weischer et al. 2014). Since this is the first large-scale study on telomere length in children there are no direct comparisons. However the rate of telomere attrition is greater in men (De Meyer et al. 2007) and it may be therefore that boys have longer telomeres and then suffer a greater subsequent loss over the course of their lives. However the children in our study are unusual in being constantly exposed to high levels of air pollution, thus the observed telomere length may be related to this exposure and may not reflect telomere characteristics in more normal circumstances.

Potential mechanisms underlying the positive association between exposure to pollution and telomere length

DNA from saliva samples is derived mainly from peripheral blood leucocytes (Thiede et al. 2000). One mechanism to explain increased leukocyte telomere length in children exposed to pollution may be that an inflammatory response in the lungs leads to recruitment of circulating leucocytes and that oxidative stress, also related to pollution, results in apoptosis or cell death. Circulating leucocytes are then replaced with cells from the bone marrow at an earlier stage of differentiation, which because they have undergone fewer cell divisions, have longer telomeres. Previous studies have shown a direct relationship between proliferative potential of hematopoietic cells and telomere length, such that early progenitors have longer telomeres than terminally differentiated cells (Thiede et al. 2000). Longer telomeres during periods of severe oxidative stress have previously been observed in adults where telomere lengths subsequently normalised when the oxidative stress was removed (Shlush et al. 2011).

Another explanation could be that exposure to pollution induces telomerase which leads to increased telomere length. However granulocytes, which form the major proportion of circulating leucocytes, have very low telomerase activity (Weng 2001). It is possible that in childhood and early life the inflammatory effects of particulate matter are most important, whereas in later life the effects of oxidative stress on telomere attrition tend to dominate, as defences against oxidative stress attenuate. Whether this effect is compounded in adults by depletion of the lymphocyte pool and a limited capacity to replace cells damaged by oxidative stress is not known.

Strengths and weaknesses

Our study is to our knowledge the largest to date examining the effects of air pollution on telomere length, and the first to examine associations in children. The study is also the first to explore fully the effects of ethnicity on telomere length in children. The children in our study are likely to be representative of the global population with children from three major continents Europe, Africa and Asia. We also used a genomic measure of continental ancestry, which confirmed the effects of self-reported ethnicity on telomere length. This genomic measure also allows a degree of quantification of continental ancestry, which means that children reporting mixed ethnicity could be included in this analysis. Since ethnicity is a major determinant of telomere length, failure to account for genetic admixture in participants may have been a problem in previous studies (Mitchell et al. 2014).

We considered that environmental tobacco smoke, obesity and level of deprivation might modify the relationship between air pollution and telomere length however in our population these factors were unrelated to the outcome so were not included in the models. We adjusted models for IgA and cortisol to address potential confounding because of a strong *a priori* hypothesis that they would be related to telomere length.

Other sources of confounding which we are not able to account for are also possible, for example level of physical activity (Ornish et al. 2008). Height or body size could potentially confound the observed inverse relationship between telomere length and lung function since height is directly related to FEV1 and children with greater body size, who have necessarily experienced a greater number cell divisions, could potentially have shorter telomeres. There is some evidence to support this hypothesis from other species (Ringsby et al. 2015) although a paucity of existing data in children. We report crude FEV1 results rather than adjusted for height as this measure is generally preferred in children because of the greater variability in

the latter. The cross sectional design of our study in pre pubertal children minimises the effects of sexual dimorphism in body habitus related to higher oestrogen and testosterone levels.

Children were selected for telomere analysis on the basis of the volume of sample available. Whilst baseline characteristics of these children are comparable to those for whom sample volume was insufficient, we cannot be certain that telomere lengths were also similar.

Implications for future research

Future studies examining associations between air pollution and telomere length should account fully for effects of ethnicity. This may either be done by considering self-reported ethnicity or by using genomic methods to assign quantitative components of continental ancestry to each participant. The latter may be achieved either by using a panel of random genetic markers as described here or by using known ancestry informative markers. Interestingly, random markers perform well against sets of individually informative markers (Pardo-Seco et al. 2014) and offer the advantage that they make no presupposition about the continent of origin of study participants. There is also the potential problem that ancestry informative markers may be directly linked with disease or critical biological pathways for example the chemokine receptor CCR5 (Galvani and Slatkin 2003). Such associations may occur because of localised evolutionary pressure on different continents. Whilst such markers may be useful for forensic purposes, the link to disease and metabolic pathways may limit their application in biological studies. The random markers that we used were selected for their lack of known clinical associations.

Conclusion

Our studies are the largest conducted so far examining telomere length in children in relation to exposure to air pollution. Since the baseline rate of loss is known to be greater in children (Aubert and Lansdorp 2008), it is possible that children may be particularly susceptible to the effects of environmental factors making this is an important area for further research. Early exposure to pollution may thus have important effects on health in later life with consequences for ageing and immunological senescence. Longitudinal studies would be necessary to determine whether the children most affected by pollution exposure, who experienced telomere lengthening in our study, will go on to have shorter leukocyte telomeres in later life with attendant increased risk of chronic diseases.

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Box 1. Linear mixed models used to examine associations between telomere length and characteristics of the children

Abbreviations:

EtBl	Reported ethnicity: Black
EtWh	Reported ethnicity: White
EtMi	Reported ethnicity: Mixed/Other
BMI	Body mass index
ETS	Environmental tobacco
IMD	Deprivation score
PAs	Proportion of genetic ancestry: Asian
PAf	Proportion of genetic ancestry: African
AP	air pollutant exposure (each of NOx, NO2, PM2.5, PM10 measures annually, over previous week and over previous day)

i… indicator for child

j… indicator for school

u_j… random effect, $u_j \sim N(0,\sigma^2_0)$

ϵ_{ij}…error term, $\epsilon_{ij} \sim N(0, \sigma^2_e)$

σ^2_0…between school variance

σ^2_e…within school variance

Model 1:

$$\log_e relTS_{ij} = \beta_0 + \beta_1 * sex_{ij} + \beta_2 * EtBl_{ij} + \beta_3 * EtWh_{ij} + \beta_4 * EtMi_{ij} + \beta_5 * BMI_{ij} + \beta_6 * ETS_{ij} + \beta_7 * IMD_{ij} + \beta_8 * age_{ij} + \beta_9 * IgA_{ij} + \beta_{10} * Cortisol_{ij} + \beta_{11} * Year2_{ij} + \beta_{12} * Year3_{ij} + u_j + \epsilon_{ij}$$

Model 2:

$$\log_e relTS_{ij} = \beta_0 + \beta_1 * sex_{ij} + \beta_2 * PAs_{ij} + \beta_3 * PAf_{ij} + \beta_4 * age_{ij} + \beta_5 * IgA_{ij} + \beta_6 * Cortisol_{ij} + \beta_7 * Year2_{ij} + \beta_8 * Year3_{ij} + u_j + \epsilon_{ij}$$

Model 3:

$$\log_e relTS_{ij} = \beta_0 + \beta_1 * sex_{ij} + \beta_2 * EtBl_{ij} + \beta_3 * EtWh_{ij} + \beta_4 * EtMi_{ij} + \beta_5 * PostFEV1_{ij} + \beta_6 * age_{ij} + \beta_7 * IgA_{ij} + \beta_8 * Cortisol_{ij} + \beta_9 * Year2_{ij} + \beta_{10} * Year3_{ij} + u_j + \epsilon_{ij}$$

Model 4 – 15

$$\log_e relTS_{ij} = \beta_0 + \beta_1 * AP_{ij} + u_j + \epsilon_{ij}$$

Model 16-27:

$$\log_e relTS_{ij} = \beta_0 + \beta_1 * AP_{ij} + \beta_2 * sex_{ij} + \beta_3 * EtBl_{ij} + \beta_4 * EtWh_{ij} + \beta_5 * EtMi_{ij} + \beta_6 * age_{ij} + \beta_7 * IgA_{ij} + \beta_8 * Cortisol_{ij} + \beta_9 * Year2_{ij} + \beta_{10} * Year3_{ij} + u_j + \epsilon_{ij}$$

496 **Table 1. Characteristics of study population**

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Variable	N obs	
Age (years), Mean(SD)	333	8.85 (0.33)
Female, N (%)	333	149 (44.7%)
Height (cm), Mean(SD)	333	133.67 (6.54)
Weight (kg), Mean(SD)	332	32.56 (8.11)
Body mass index (kg/m^2), Mean(SD)	332	18.04 (3.49)
International Obesity Task Force Grade, N (%)	332	
-2		4 (1.2%)
-1		21 (6.3%)
0		206 (61.9%)
1		62 (18.6%)
2		39 (11.7%)
Index of multiple deprivation score, Mean (SD)	332	44.6 (12.7)
Reported ethnicity, N (%)	333	
Asian		128 (38.4%)
Black		73 (21.9%)
White		88 (26.4%)
Mixed/Other		44 (13.2%)
Genetic ancestry components, Mean (SD)	327	
Asian		0.39 (0.29)
African		0.23 (0.35)
European		0.38 (0.28)
Environmental tobacco smoke exposure, N (%)	324	61 (18.8%)
Ig A (ng/ml), Mean(SD)	330	53700.94 (60149.12)
Cortisol, Mean(SD)	315	864.89 (1385.73)
Post bronchodilator FEV1 (L) d	327	1.68 (0.28)
Annual pollution exposures ($\mu g/m^3$)	333	
NO_x		76.12 (16.16)
NO_2		43.59 (5.93)
$PM_{2.5}$		13.70 (0.82)
PM_{10}		23.36 (1.53)

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Table 2. Associations between telomere length, ethnicity and lung function

	Ratio of geometric means [95% CI]		
Measure	**Model 1 (N=305)**	**Model 2 (N=309)**	**Model 3 (N=309)**
Female vs Male	0.917 [0.857,0.982]*	0.923 [0.863,0.987]**	0.914 [0.853,0.979]*
Reported ethnicity (Reference: Asian)			
Black	1.224 [1.104,1.358]**	-	1.222 [1.101,1.355]**
White	0.987 [0.892,1.093]	-	0.983 [0.893,1.083]
Mixed/Other	0.999 [0.889,1.123]	-	1.011 [0.900,1.137]
Body mass index	0.996 [0.986,1.006]	-	-
Environmental tobacco	1.000 [0.998,1.001]	-	-
Deprivation score	1.001 [0.998,1.004]	-	-
Continental ancestry (genomic markers)			
Asian	-	0.897 [0.769,1.046]	-
African	-	1.161 [1.014,1.330]*	-
Post bronchodilator FEV1 (L)	-	-	0.898 [0.792,1.019]

* $p<0.05$, ** $p<0.001$

The models were also adjusted for age, Ig A, cortisol and study year and included a random intercept for school

505 **Table 3. Associations between pollution exposure and telomere length (model**
506 **coefficient [95% CI]).**

	Ratio of geometric means		
	Crude [95% CI]	**Adjusted# [95% CI]**	**difference between 25th and 75th centile of exposure**
Measure	**Models 4 – 15 (N=333)**	**Models 16 – 27 (N=315)**	
Annual air pollution exposure (μg/m^3)			
NOx	1.003 [1.001,1.005]*	1.004 [1.002,1.006]**	1.016
NO_2	1.007 [1.001,1.013]*	1.012 [1.005,1.016]**	1.044
$PM_{2.5}$	1.007 [0.963,1.052]	1.116 [1.056,1.179]**	1.124
PM_{10}	1.026 [1.004,1.049]*	1.047 [1.024,1.071]**	1.063
Exposure over previous week			
NOx	1.004 [1.001,1.006]*	1.003 [1.000,1.006]*	1.052
NO_2	1.008 [1.002,1.015]*	1.007 [1.000,1.014]	-
$PM_{2.5}$	1.016 [1.004,1.028]*	1.013 [1.000,1.025]*	1.051
PM_{10}	1.013 [1.005,1.020]**	1.010 [1.002,1.018]*	1.053
Exposure over previous day			
NOx	1.000 [0.999,1.002]	1.001 [0.999,1.002]	-
NO_2	1.002 [0.997,1.006]	1.001 [0.997,1.006]	-
$PM_{2.5}$	1.003 [0.994,1.011]	1.004 [0.995,1.014]	-
PM_{10}	1.002 [0.997,1.008]	1.003 [0.997,1.009]	-

507 * $p<0.05$, ** $p<0.001$
508 # The models were adjusted for age, sex, ethnicity, study year, IgA, cortisol and included a
509 random intercept for school.

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Supplementary Table 1

		Telomere data	
		not available	**available**
Age	Mean (SD)	N=779, 8.8 (0.3)	N=333, 8.9 (0.3)
Height	Mean (SD)	N=680,133.8 (6.8)	N=333, 133.7 (6.5)
Weight	Mean (SD)	N=680, 32.5 (7.8)	N=332, 32.6 (8.1)
BMI	Mean (SD)	N=680, 18.0 (3.2)	N=332, 18.0 (3.5)
IMD score	Mean (SD)	N=776, 45.9 (10.4)	N=332, 44.6 (12.7)
Study year		N=779	N=333
	Year 1	202 (26%)	0 (0%)
	Year 2	334 (43%)	117 (35%)
	Year 3	243 (31%)	216 (65%)
Ethnicity		N=765	N=333
	Asian	275 (36%)	128 (38%)
	Black	197 (26%)	73 (22%)
	White	209 (27%)	88 (26%)
	Other	84 (11%)	44 (13%)
Gender		N=762	N=333
	Male	376 (49%)	184 (55%)
	Female	386 (51%)	149 (45%)
Asthma	N(%)	104/779 (13%)	39/333 (12%)
Environmental tobacco exposure	N(%)	157/646 (24%)	61/324 (19%)
Annual air pollution exposure (µg/m^3)			
NOx	Mean (SD)	N=775, 75.2 (13.4)	N=333, 76.1 (16.2)
NO_2	Mean (SD)	N=775, 43.4 (5.1)	N=333, 43.6 (5.9)
$PM_{2.5}$	Mean (SD)	N=775, 13.7 (0.8)	N=333, 13.7 (0.8)
PM_{10}	Mean (SD)	N=775, 23.4 (1.4)	N=333, 23.4 (1.5)

517 **Figure 1. Flow of participants through the study**

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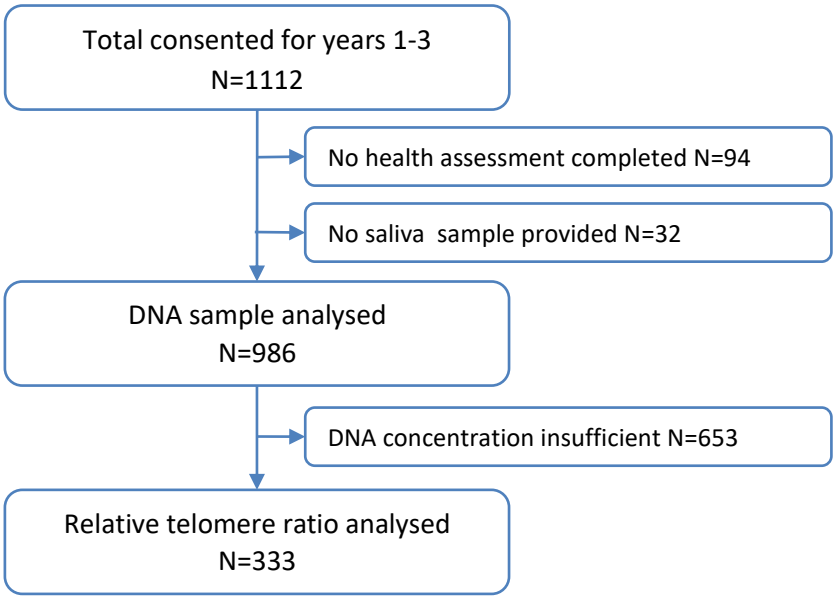

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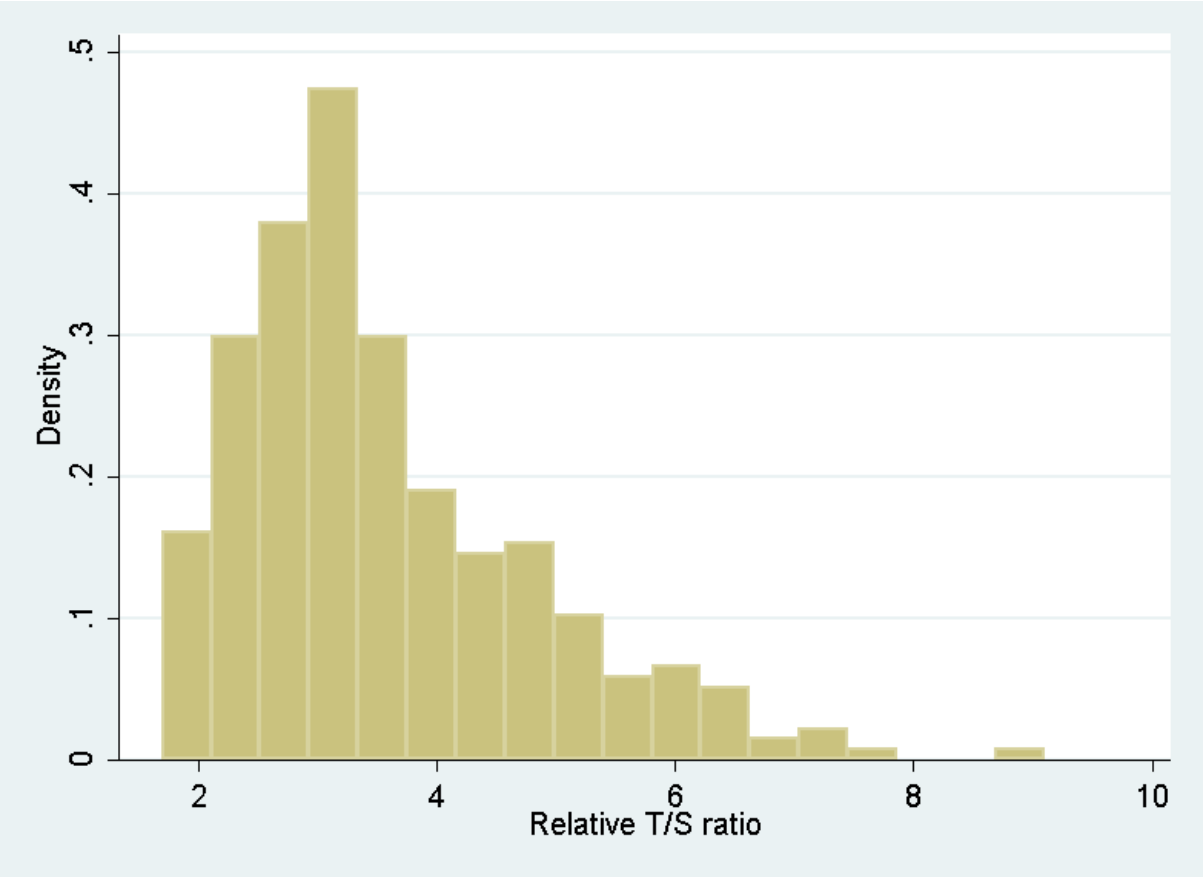
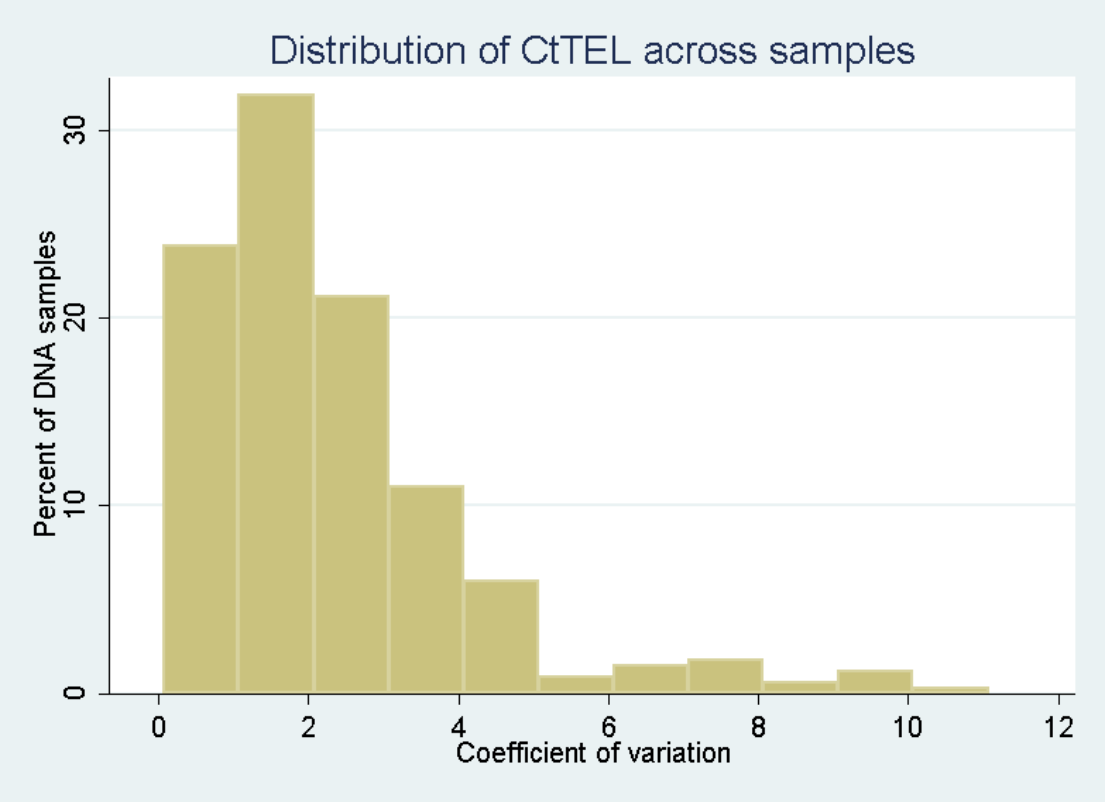
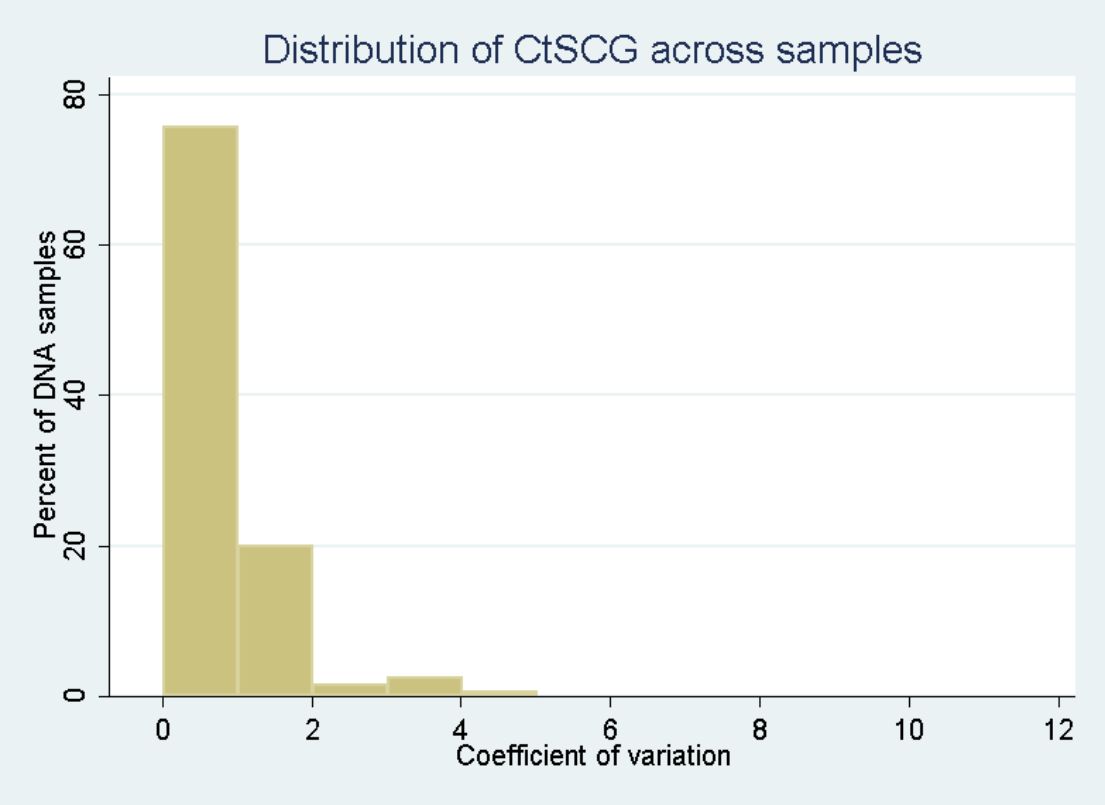
Supplementary Figure 1. Frequency distribution of relative telomere length (T/S ratio)

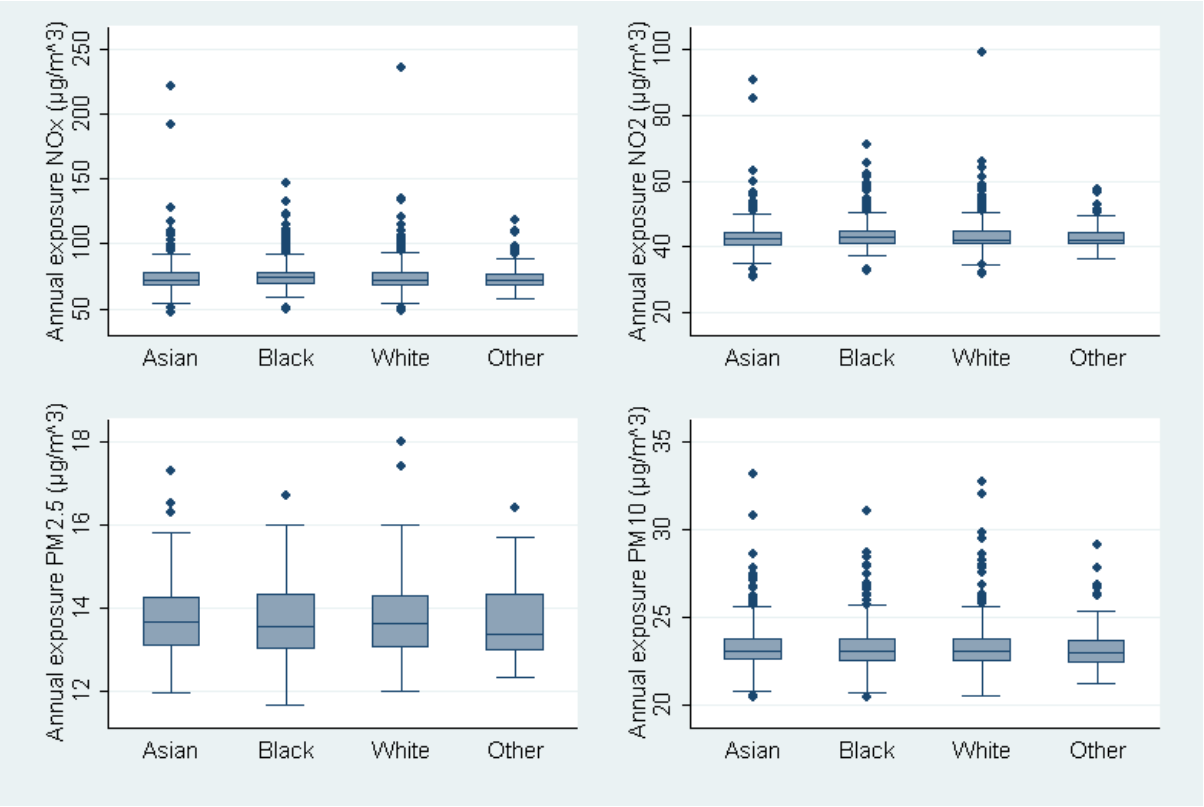


Supplementary Figure 2. Frequency distribution of the coefficient of variation for telomere and single copy gene determinations





Supplementary Figure 3. Annual pollution exposure by reported ethnicity



Supplementary Figure 4. Annual pollution exposure by proportion of African ancestry.