EUROPEAN RESPIRATORY *journal*

FLAGSHIP SCIENTIFIC JOURNAL OF ERS

Early View

Original article

Genetic susceptibility to asthma increases the vulnerability to indoor air pollution

Anke Hüls, Aneesa Vanker, Diane Gray, Nastassja Koen, Julia L. MacIsaac, David T.S. Lin, Katia E. Ramadori, Peter D. Sly, Dan J. Stein, Michael S. Kobor, Heather J. Zar

Please cite this article as: Hüls A, Vanker A, Gray D, *et al*. Genetic susceptibility to asthma increases the vulnerability to indoor air pollution. *Eur Respir J* 2020; in press (https://doi.org/10.1183/13993003.01831-2019).

This manuscript has recently been accepted for publication in the *European Respiratory Journal*. It is published here in its accepted form prior to copyediting and typesetting by our production team. After these production processes are complete and the authors have approved the resulting proofs, the article will move to the latest issue of the ERJ online.

Journal: European Respiratory Journal

Title

Genetic susceptibility to asthma increases the vulnerability to indoor air pollution

Anke Hüls PhD[1,2], Aneesa Vanker PhD[3], Diane Gray PhD[3], Nastassja Koen PhD[4,5], Julia L MacIsaac PhD[2], David TS Lin PhD[2], Katia E Ramadori BS[2], Peter D Sly PhD[6], Dan J Stein PhD[4,5], Michael S Kobor PhD[2], Heather J Zar PhD[3]

[1]Department of Human Genetics, Emory University, Atlanta, Georgia, USA

[2]Centre for Molecular Medicine and Therapeutics, BC Children's Hospital Research Institute, and Department of Medical Genetics, University of British Columbia, Vancouver, British Columbia, Canada

[3]Department of Paediatrics and Child Health, Red Cross War Memorial Children's Hospital and SA-MRC Unit on Child and Adolescent Health, University of Cape Town

[4]Department of Psychiatry and Mental Health, University of Cape Town, South Africa

[5]South African Medical Research Council (SAMRC) Unit on Risk and Resilience in Mental Disorders

[6]Children's Health and Environment Program, Child Health Research Centre, The University of Queensland, Brisbane, Queensland, Australia.

Corresponding author:

Anke Hüls, PhD

Department of Human Genetics, Emory University

Whitehead Biomedical Research Building

615 Michael St

Atlanta, GA 30322, USA

Email: anke.huels@emory.edu

Phone: +1 (404) 727 5326

Authors' contribution: AH planned and conducted the analyses and was the major contributor in writing the manuscript. PDS supervised the collection and interpretation of air pollution and lung function data. JLM, DTSL, and KER performed the microarray experiments, which were supervised by MSK. HJZ conceived, designed and leads the Drakenstein child health study (DCHS) and obtained funding; DG led and was responsible for the lung function aspects; AV led and was responsible for the IAP and ETS measurements; NK oversaw work on genetics testing in the (DCHS); DJS conceived, designed and led the genetics aspects of the DCHS. All authors contributed to the final paper.

"Take home" message

Our findings from a South African birth cohort study show an association of indoor air pollution with reduced lung function at six weeks and at one year of age with a higher susceptibility in children with a genetic predisposition for asthma.

Abstract

Introduction: Indoor air pollution and maternal smoking during pregnancy are associated with respiratory symptoms in infants, but little is known about the direct association with lung function or interactions with genetic risk factors. We examined associations of indoor

particulate matter of diameter ≤10μm (PM_{10}) exposure and maternal smoking with infant lung function and the role of gene-environment interactions.

Methods: Data from the Drakenstein Child Health Study, a South African birth cohort, were analyzed (N=270). Lung function was measured at 6 weeks and 1 year of age and lower respiratory tract illness (LRTI) episodes were documented. We measured prenatal and postnatal PM_{10} exposures using devices placed in homes and prenatal tobacco smoke exposure using maternal urine cotinine levels. Genetic risk scores (GRS) determined from associations with childhood-onset asthma (COA) in the UK Biobank were used to investigate effect modifications.

Results: Pre- and postnatal exposure to PM_{10} as well as maternal smoking during pregnancy were associated with reduced lung function at 6 weeks and 1 year as well as LRTI in the first year. Due to a significant interaction between the GRS and prenatal exposure to PM_{10}, infants carrying more COA-risk alleles were more susceptible to PM_{10}-associated reduced lung function (p-interaction=0.007). This interaction was stronger in infants with black African ancestry (p-interaction=0.001) and non-existent in children with mixed ancestry (p-interaction=0.876).

Conclusions: PM_{10} and maternal smoking exposures were associated with reduced lung function, with a higher susceptibility for infants with an adverse genetic predisposition for asthma that also depended on the infant's ancestry.

Funding:

The Drakenstein Child Health Study was funded by the Bill & Melinda Gates Foundation, Discovery Foundation, Medical Research Council South Africa, National Research Foundation South Africa, CIDRI Clinical Fellowship and Wellcome Trust (204755/2/16/z). AH received a

research fellowship from the Deutsche Forschungsgemeinschaft (DFG; HU 2731/1-1). The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of manuscript. DJS,HJZ and NK are supported by the SA Medical Research Council (SAMRC). PDS is supported by the National Health and Medical Research Council, Australia.

Key words: early life, gene-environment interactions, weighted genetic risk scores, polygenic risk scores

Introduction

Indoor air pollution and tobacco smoke exposure are important risk factors for child health with infants being a highly susceptible subgroup (1). According to the Global Burden of Disease, Injuries, and Risk Factors Study 2015, air pollution is the biggest environmental cause of death worldwide, with indoor air pollution accounting for about 2.9 million deaths and 85.6 million disability-adjusted life-years (DALYs) in 2015 (2). Indoor air pollution arises from domestic activities of cooking, heating, and lighting, particularly in low and middle income countries (LMICs). Three billion people worldwide are exposed to toxic amounts of indoor air pollution every day because they use solid fuels, a term that includes biomass fuels (derived from plant sources) or coal for combustion (1).

Similarly, tobacco smoke exposure is a major risk for poor health. Despite worldwide initiatives to reduce tobacco smoking, it is estimated that up to 40% of children are still exposed to environmental tobacco smoke (3). In LMICs the incidence of smoking is increasing, especially amongst women of child bearing age, which leads to a high tobacco smoke exposure in children (4,5).

Indoor air pollution and tobacco smoke have been strongly associated with the development of childhood respiratory illness, particularly with lower respiratory tract infection (LRTI) or wheezing (1,6). Lung development and maturation is incomplete at birth and continues during the first years of life making lungs particularly vulnerable to damage during this critical time of lung development.

Recently, we showed that prenatal rather than postnatal exposure to indoor air pollution and tobacco smoke was associated with LRTI and wheezing in infants from the Drakenstein Child Health Study (DCHS), a South African birth cohort study, which highlights the importance of the timing of exposure on child respiratory health (6). The precise mechanisms are still largely unclear, but it is hypothesized that prenatal exposure might act directly on the developing fetus

or induce a systemic immune or inflammatory response. This inflammatory response might subsequently lead to placental insufficiency and reduced supply of oxygen and nutrients to the fetus (7,8).

Asthma is a spectrum of airways disease (9) that is highly heritable, and associated with environmental exposures including air pollution or tobacco smoke (10). A recent genome-wide association study of data from the UK Biobank study (13,962 cases and 300,671 controls) showed that a large extent of the variance in the liability of paediatric asthma is explained by common genetic variants (onset at ages between 0 and 19 years; h^2=25.6%) with 123 independent SNPs being genome-wide significant (11). Beside the direct associations with air pollution or tobacco smoke and genetic risk factors, evidence from toxicological and gene-environment studies further suggests that the association is particularly pronounced in genetically predisposed individuals (12,13). However, if and how a genetic predisposition for asthma also affects early lung development and its susceptibility to environmental exposures remains unclear.

In this study we used data from children in the DCHS to investigate associations of pre- and postnatal exposure to indoor air pollution (PM_{10}) or maternal smoking during pregnancy with infant lung function at 6 weeks and 1 year of age and effect modifications by a genetic predisposition for asthma.

Methods

Study design and study population

The DCHS, a population-based birth cohort, has been described previously (14–16).

Infants enrolled in the DCHS were followed from birth until at least one year of age (14). Mothers were enrolled prenatally in their second trimester and followed through pregnancy at two primary care clinics serving two distinct populations (predominantly black African ancestry

or predominantly mixed ancestry). Mother-child pairs were followed from birth. All births occurred at a single, central facility, Paarl Hospital. Infants attended scheduled study visits at 6, 10, and 14 weeks and 6, 9, and 12 months of age, with lung function assessed at 6 weeks and 1 year.

The sample included in the present study were 270 children who had all measurements available including lung function at 6 weeks and 1 year of age and genotyping data.

Lung function testing was undertaken at 6 (5–11) weeks of age corrected for prematurity (<37 weeks) and then at 1 year (11–13 months). All testing was done in unsedated, behaviorally assessed quiet sleep as previously described (15,17,18). Infant lung function variables assessed were functional residual capacity (FRC) and tidal volume, as measures of early lung growth (15). Study staff trained in the recognition of LRTI documented all episodes, either ambulatory or hospitalised. We defined LRTI using WHO case definition criteria (6).

Pregnant women were enrolled at 20–28 weeks' gestation and a prenatal (within 4 weeks of enrolment) and postnatal (between 4 and 6 months of the infant's life) home visit was undertaken to assess the home environment and measure indoor air pollution. Particulate matter of diameter 10 μm or less (PM_{10}) was measured using a personal air sampling pump (AirChek 52; SKC, Eighty Four, PA, USA), connected to a cassette (SKC 37mm cassette blank – 225-3050LF) with a gravimetrically pre-weighed filter (SKC PVC Filter 37x5 + Pad 225-8-01) (19) left in homes for 24 h (6,16). Filters were weighed post sampling and analysed using NIOSH method 0600 to obtain an average concentration over 24 hours (20). These average concentrations over 24 hours were used as exposures in the analyses. Exposure to maternal tobacco smoke was assessed by prenatal urine cotinine measures in mothers. Urine cotinine was measured using the IMMULITE® 1000 Nicotine Metabolite Kit (Siemens Medical Solutions Diagnostics®, Glyn Rhonwy, United Kingdom). This provides a quantitative test using a competitive chemiluminescent immunoassay, which contained solid-phase beads coated with

polyclonal rabbit anti-cotinine antibody. Quantitative analyses were used to classify exposure as <10 ng/ml (non-smoker), 10-499 ng/ml, (passive smoker/exposed), or ≥500 ng/ml (active smoker) (6).

Assessment of genotypes

Offspring samples were selected for genotyping analysis based on a number of criteria relevant to the DCHS as a whole – including (but not limited to) maternal psychosocial risk/stressors and/or availability of offspring lung function data. DNA was isolated from cord blood samples that were collected at time of delivery (21). Genome-wide genotyping was performed in 270 newborns using the Illumina Infinium PsychArray (n=119) and the Illumina Infinium Global Screening Array, GSA (n=151). After quality control (QC) SNPs were imputed on the 1000 Genomes reference panel (Phase III) using the Michigan Imputation Server (22). Imputed genotypes reaching an $R^2 \geq 0.3$ in both arrays were used in analyses. Principal components were calculated in the whole study sample using PLINK v1.90b4 64-bit (23,24).

Statistical analysis

We investigated the association between prenatal and postnatal exposure to PM_{10} and maternal smoking during pregnancy with lung function at 6 weeks and 1 year of age in adjusted linear models and with LRTI in adjusted logistic models. *A priori* selected covariates which could potentially act as confounders included sex, birth weight (kg), age for weight z-scores at birth based on Fenton Growth Chart, maternal HIV status, ancestry of child, age and height at time of lung function measurement and socio economic status (SES) quartile. Associations with PM_{10} were additionally adjusted for maternal smoking behavior during pregnancy based on prenatal urine cotinine measures. In a sensitivity analysis, associations with lung function were

further adjusted for LRTI in the first year. All associations including genotyping data were additionally adjusted for genotying array (Illumina Infinium PsychArray or Illumina Infinium Global Screening Array, GSA) and for the first five principal components to correct for population stratification (see Supplementary Figure S1).

To investigate the impact of genetic factors on the association between PM_{10} or maternal smoking during pregnancy with early lung growth, we summarized the genetic susceptibility to childhood-onset asthma in a genetic risk score (GRS), which was calculated as follows, and estimated its interaction with PM_{10} or maternal smoking during pregnancy on newborn lung growth in a linear regression analysis: The GRS was based on summary statistics from a recent GWAS study of childhood-onset asthma in the UK Biobank (13,962 cases and 300,671 controls) (11) and was calculated using PRSice (25). PRSice calculates the sum of alleles weighted by their effect sizes estimated from a GWAS of that phenotype in an independent sample (here from (11)) using an approach called clumping and thresholding. Clumping was used to obtain SNPs in linkage equilibrium with an $r^2 < 0.1$ within a 250 bp window, keeping the SNP with the lower p-value observed in the external dataset, for the analysis (see Tables S1 and S2 for a list of SNPs that were included in the construction of the GRS after clumping and thresholding, summary statistics are published under https://genepi.qimr.edu.au/staff/manuelF/gwas_results/main.html (last access 2019-11-08)). Multiple scores were then created for containing SNPs selected according to the significance of their association with the phenotype. As proposed in (25), the GRS that predicts the specific lung function parameter best (highest R^2) was used for analysis (Table S3). Since the distribution of GRS strongly depends on ancestry (26), GRS were calculated in ancestrally homogeneous subgroups (black African vs. mixed ancestry) adjusted for sex, genotyping array and the first five principal components. The resulting GRS were standardized (z-scores) to

control for ancestry-specific differences in distribution before the data from both ancestries was merged for the subsequent interaction analyses.

Results

Description of study participants

The characteristics of the study participants (N=270 infants) are described in Table 1. Around half of the infants were female (45%) and the infants were almost equally distributed between two population groups (56% black African, 44% mixed ancestry). A high percentage of mothers were HIV-infected (24%) and there were many active smokers amongst pregnant women, as well as high rates of passive smoke exposure, as determined by maternal cotinine measurements. Approximately one third of the children had at least one LRTI in the first year of life.

Due to the skewed distribution of PM_{10} exposure levels (Figure S2), exposure levels were log-transformed prior to analyses. Prenatal PM_{10} levels were slightly higher than postnatal PM_{10} levels (difference not significant).

The study characteristics of our study population (N=270 samples with genotype data) were very similar to the characteristics of the whole study population (N=1143, Table 1).

PM_{10} and maternal smoking associated with reduced infant lung function and LRTI

Prenatal exposure to PM_{10} was associated with reduced FRC at the age of 1 year (Table 2; β-estimate (95%-CI): -9.0 (-17.2; -0.9) per increase of IQR) and postnatal exposure to PM_{10} with reduced tidal volume at 1 year (Table 2; (β-estimate (95%-CI): -2.9 (-5.4; -0.5) per increase of IQR). Maternal smoking during pregnancy was associated with a -2.4 ml (95%-CI: -4.7; -0.1) lower tidal volume at the age of 6 weeks as well as with higher odds for LRTI in the first ywar

of life (Table 2). Associations with FRC and tidal volume were not confounded by LRTI (Table S4).

Infants with genetic predisposition to asthma more susceptible to prenatal PM_{10} exposure

Altough the GRS was not directly associated with lung function at 6 weeks or 1 year of age (Tables S3 and S5), we found a significant gene-environment interaction between the GRS and prenatal PM_{10} on FRC at 6 weeks of age (p-value interaction = 0.007; Table 3). The direction of interaction was consistent for FRC and tidal volume at 6 weeks as well as 1 year of age, with infants with a higher GRS being more susceptible to prenatal PM_{10} exposure, which corresponds to carrying more asthma-related risk alleles (Figure 1). No interactions were found for LRTI.

Genetic susceptibility to prenatal PM_{10} exposure depends on ancestry

The genetic susceptibility to PM_{10} strongly depended on the infant's ancestry: We found a strong gene-environment interaction with prenatal PM_{10} exposure in infants with black African ancestry, which was significant for FRC and tidal volume at 6 weeks as well as for tidal volume at 1 year, whereas no interactions were found for infants with mixed ancestry (Table 3 and Figure 1).

Discussion

In this study of infants from a poor peri-urban community in South Africa, we showed that pre- and postnatal exposure to PM_{10} as well as maternal smoking during pregnancy were associated with reduced lung function at the age of six weeks and one year. Of note, these associations were altered by genetic risk factors and ancestry: Infants with a genetic predisposition for

asthma were more susceptible to the adverse effects of prenatal PM_{10}, and this interaction also depended on the infants' ancestry with infants of black African ancestry being more vulnerable. Longitudinal cohort studies have shown that lung function trajectories are set in early life with a developmental window of susceptibility, which can be disrupted by both infectious and environmental exposures (27–29). The present study provided evidence that early exposure to PM_{10} and maternal smoking during pregnancy not only affect susceptibility to develop childhood LRTI or wheezing (6), but are also directly associated with infant lung function at the age of 6 weeks as well as at 1 year. Consequently, prenatal exposures to indoor PM_{10} and maternal smoking during pregnancy might be risk factors for reduced airflow and COPD later in life (30,31).

We recently reported in this cohort that the timing of exposure was crucial to the susceptibility to childhood LRTI or wheezing (6); exposure during pregnancy was an even stronger risk factor for respiratory diseases in early life rather than postnatal exposure. This is consistent with data from the present study, in which we found stronger associations with prenatal exposures, especially in interaction with a genetic predisposition for asthma. In our study, not every infant exposed to high levels of prenatal PM_{10} had the same risk for reduced lung function: Our gene-environment interaction analyses revealed that infants who carry more asthma-related risk alleles were more susceptible to PM_{10}-associated reduced lung function.

As of now little is known about the genetic susceptibility to environmental pollutants and their association with lung function and most evidence is based on samples of European ancestry. Asthma is one of the few respiratory outcomes with robust evidence that associations with environmental exposures can be altered by genetic risk factors (12,13). This evidence is mainly based on a candidate SNP study of 5,115 children (12) as well as a genome-wide interaction analysis (~1,500 children in discovery cohort and ~1,800 children in replication cohort) (13). In addition, some studies have shown the relevance of gene-environment interactions with

ambient air pollution (32), occupational exposures (33) as well as smoking (34) for adult lung function. Our current study extends this evidence by showing the importance of gene-environment interactions for infant lung function in a non-European cohort.

Strengths & Limitations

Strengths of this study include the longitudinal follow-up, prospective collection of data, high cohort retention, and repeated objective measures of indoor air pollution through the prenatal period and through infancy. A further strength was the carefully conducted lung function measurements, which were consistently assessed by the same investigators, using the same testing techniques. Lung function was undertaken in a community-based cohort with robust LRTI surveillance and simultaneous measurement of comprehensive risk factors for impaired lung growth. The advantage of using lung function measurements in addition to the broad clinical definition of LRTI or reported wheezing is the continuous data structure and objective measurements, which provide comprehensive measurements of lung health.

Limitations of the study are the potential lack of generalizability to other settings with different exposures; however, many of these exposures are common in LMIC settings. Another limitation is the small sample size with genotype data (n=270). However, by using polygenic risk score approaches, we reached a sufficient power to detect interaction effects (35,36). A limitation of our risk score approaches was the validity of the external reference populations: We used a GRS based on samples from European ancestry, which often have a lower predictive performance in non-European ancestry samples (26). Furthermore, very little is known about the performance of GRS in populations of mixed ancestry, who are amongst the most genetically diverse populations globally. In our study, 44% of the infancts were of mixed ancestry. In this subgroup, we could not find any gene-environment interactions, which might

have at least two possible reasons: Either, infants with black African ancestry and a genetic predisposition for asthma are more prone to the harmful effects of indoor air pollution than infants of mixed ancestry or the GRS was simply not applicable for infants of mixed ancestry, which is a very heterogenous subgroup with genotypes of varying minor allele frequencies (see PCA plot in Figure S1). More research is needed on GRS approaches for populations of mixed ancestry.

Conclusions

Pre- and postnatal exposure to PM_{10} and maternal smoking during pregancny were associated with reduced lung function at six weeks and one year. We further identified infants with a genetic predisposition for asthma as being a highly susceptible subgroup for the adverse effects of prenatal exposure to indoor air pollution, which highlights the importance of gene-environment interactions for infant lung function.

Acknowledgments:

The authors thank the study and clinical staff at Paarl Hospital, Mbekweni and TC Newman clinics, as well as the CEO of Paarl Hospital, and the Western Cape Health Department for their support of the study. The authors thank the families and children who participated in this study.

Competing financial interests declaration:

All authors declare they have no actual or potential competing financial interest.

References

1. Gordon SB, Bruce NG, Grigg J, Hibberd PL, Kurmi OP, Lam K-BH, et al. Respiratory risks from household air pollution in low and middle income countries. Lancet Respir Med. 2014 Oct;2(10):823–60.
2. GBD 2015 Risk Factors Collaborators. Global, regional, and national comparative risk assessment of 79 behavioural, environmental and occupational, and metabolic risks or clusters of risks, 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet. 2016 Oct 8;388(10053):1659–724.
3. Oberg M, Jaakkola MS, Woodward A, Peruga A, Prüss-Ustün A. Worldwide burden of disease from exposure to second-hand smoke: a retrospective analysis of data from 192 countries. Lancet. 2011 Jan 8;377(9760):139–46.
4. World Health Organisation. WHO report on the global tobacco epidemic. WHO Press. 2013;
5. Samet J, Yoon S. Gender, women, and the tobacco epidemic. WHO Press Geneva. 2010;
6. Vanker A, Barnett W, Workman L, Nduru PM, Sly PD, Gie RP, et al. Early-life exposure to indoor air pollution or tobacco smoke and lower respiratory tract illness and wheezing in African infants: a longitudinal birth cohort study. Lancet Planet Heal. 2017;1(8):e328–36.
7. Korten I, Ramsey K, Latzin P. Air pollution during pregnancy and lung development in the child. Paediatr Respir Rev. 2017;21:38–46.
8. Kannan S, Misra DP, Dvonch JT, Krishnakumar A. Exposures to airbone particulate matter and adverse perinatal outcomes: A biologically plausible mechanistic framework for exploring potential effect modification by nutrition. Environ Health Perspect.

2006;114(11):1636–42.

9. Pavord ID, Beasley R, Agusti A, Anderson GP, Bel E, Brusselle G, et al. After asthma: redefining airways diseases. Lancet. 2018;391(10118):350–400.
10. Achakulwisut P, Brauer M, Hystad P, Anenberg SC. Global, national, and urban burdens of paediatric asthma incidence attributable to ambient NO 2 pollution: estimates from global datasets. Lancet Planet Heal. 2019;3(4):e166–78.
11. Ferreira MAR, Mathur R, Vonk JM, Szwajda A, Brumpton B, Granell R, et al. Genetic Architectures of Childhood- and Adult-Onset Asthma Are Partly Distinct. Am J Hum Genet. 2019;104(4):665–84.
12. MacIntyre EA, Brauer M, Melén E, Bauer CP, Bauer M, Berdel D, et al. GSTP1 and TNF gene variants and associations between air pollution and incident childhood asthma: The traffic, asthma and genetics (TAG) study. Environ Health Perspect. 2014;122(4):418–24.
13. Gref A, Merid SK, Gruzieva O, Ballereau S, Becker A, Bellander T, et al. Genome-Wide Interaction Analysis of Air Pollution Exposure and Childhood Asthma with Functional Follow-up. Am J Respir Crit Care Med. 2017 May 15;195(10):1373–83.
14. Zar HJ, Barnett W, Myer L, Stein DJ, Nicol MP. Investigating the early-life determinants of illness in Africa: The Drakenstein Child Health Study. Thorax. 2015;70(6):592–4.
15. Gray DM, Turkovic L, Willemse L, Visagie A, Vanker A, Stein DJ, et al. Lung function in African infants in the Drakenstein child health study impact of lower respiratory tract illness. Am J Respir Crit Care Med. 2017;195(2):212–20.
16. Vanker A, Barnett W, Nduru PM, Gie RP, Sly PD, Zar HJ. Home environment and indoor air pollution exposure in an African birth cohort study. Sci Total Environ [Internet]. 2015;536:362–7. Available from: http://dx.doi.org/10.1016/j.scitotenv.2015.06.136

17. Gray D, Willemse L, Visagie A, Smith E, Czövek D, Sly PD, et al. Lung function and exhaled nitric oxide in healthy unsedated African infants. Respirology. 2015;20(7):1108–14.

18. Gray DM, Willemse L, Alberts A, Simpson S, Sly PD, Hall GL, et al. Lung function in African infants: A pilot study. Pediatr Pulmonol. 2015;50(1):49–54.

19. SKC. Filters and Cassettes Selection Guide [Internet]. [cited 2019 Nov 8]. Available from: https://www.skcltd.com/knowledge-library/product-selection-guides/filters-cassettes-selection-guide.html

20. The National Institute for Occupational Safety and Health (NIOSH). NIOSH Manual of Analytical Methods (NMAM) - 4th edition, 3rd supplement [Internet]. [cited 2014 Oct 20]. Available from: http://www.cdc.gov/niosh/docs/2003-154/

21. Morin AM, Gatev E, McEwen LM, Macisaac JL, Lin DTS, Koen N, et al. Maternal blood contamination of collected cord blood can be identified using DNA methylation at three CpGs. Clin Epigenetics. 2017;9(1):1–9.

22. Das S, Forer L, Schönherr S, Sidore C, Locke AE, Kwong A, et al. Next-generation genotype imputation service and methods. Nat Genet. 2016;48(10):1284–7.

23. Purcell S, Chang C. PLINK [Internet]. 2017. Available from: www.cog-genomics.org/plink/1.9/

24. Chang CC, Chow CC, Tellier LC, Vattikuti S, Purcell SM, Lee JJ. Second-generation PLINK: rising to the challenge of larger and richer datasets. Gigascience. 2015;4:7.

25. Euesden J, Lewis CM, O'Reilly PF. PRSice: Polygenic Risk Score software. Bioinformatics. 2015;31(9):1466–8.

26. Duncan L, Shen H, Gelaye B, Meijsen J, Ressler K, Feldman M, et al. Analysis of

polygenic risk score usage and performance in diverse human populations. Nat Commun. 2019;10(1):3328.

27. Guerra S, Martinez FD. Epidemiology of the Origins of Airflow Limitation in Asthma. Proc Am Thorac Soc. 2009;6(8):707–11.

28. Martinez FD. The Origins of Asthma and Chronic Obstructive Pulmonary Disease in Early Life. Proc Am Thorac Soc. 2009;6(3):272–7.

29. Stern DA, Morgan WJ, Wright AL, Guerra S, Martinez FD, Fernando D. Poor airway function in early infancy and lung function by age 22 years: a non-selective longitudinal cohort study. Lancet. 2007;370(9589):758–64.

30. Upton MN, Smith GD, McConnachie A, Hart CL, Watt GCM. Maternal and Personal Cigarette Smoking Synergize to Increase Airflow Limitation in Adults. Am J Respir Crit Care Med. 2004;169(4):479–87.

31. Vanker A, Gie RP, Zar HJ. The association between environmental tobacco smoke exposure and childhood respiratory disease: a review. Expert Rev Respir Med. 2017;11(8):661–73.

32. Hüls A, Sugiri D, Abramson MJ, Hoffmann B, Schwender H, Ickstadt K, et al. Benefits of improved air quality on ageing lungs: impacts of genetics and obesity. Eur Respir J. 2019 Apr;53(4).

33. Liao S-Y, Lin X, Christiani DC. Gene-environment interaction effects on lung function- a genome-wide association study within the Framingham heart study. Environ Heal. 2013 Dec 1;12:101.

34. Aschard H, Tobin MD, Hancock DB, Skurnik D, Sood A, James A, et al. Evidence for large-scale gene-by-smoking interaction effects on pulmonary function. Int J Epidemiol.

2017;46(3):894–904.

35. Hüls A, Krämer U, Carlsten C, Schikowski T, Ickstadt K, Schwender H. Comparison of weighting approaches for genetic risk scores in gene-environment interaction studies. BMC Genet. 2017;18:115.

36. Aschard H. A perspective on interaction effects in genetic association studies. Genet Epidemiol. 2016 Jul 7;40(8):678–88.

Tables

Table 1. Demographics of infants used for analysis (N=270 samples with genotype information) as well as of infants from the whole study population (N=1143).

	Analysis sample (N=270)	**Whole study population (N=1143)**	**p-values**
Female, n (%)	122 (45.2%)	554 (48.5%)	0.3435
Age at 6 weeks investigation (in months), mean ± sd	1.7 ± 0.3	1.8 ± 0.4	0.0004
Age at 1 year investigation (in months), mean ± sd	12.4 ± 0.8	12.5 ± 1.0	0.1427
Black African ancestry, n (%)	151 (55.9%)	632 (55.3%)	0.8918
Mixed ancestry, n (%)	119 (44.1%)	511 (44.7%)	
GSA array for genotyping	151 (55.9%)	NA	NA
Psych Array for genotyping	119 (44.1%)	NA	
Birth weight (kg), mean ± sd	3.1 ± 0.5	3.0 ± 0.6	0.0474
Age for weight z-scores at birth based on Fenton Growth Chart, mean ± sd	-0.6 ± 1.0	-0.6 ± 1.1	0.7524
Height at 6 weeks investigation (in cm), mean ± sd	55.3 ± 3.1	55.2 ± 2.8	0.4764
Height at 1 year investigation (in cm), mean ± sd	74.0 ± 3.1	73.8 ± 3.2	0.3425
Mother HIV positive, n (%)	66 (24.4%)	248 (21.7%)	0.3708
Socio economic status high, n (%)	67 (24.8%)	283 (24.8%)	0.9270
Socio economic status moderate to high, n (%)	66 (24.4%)	290 (25.4%)	
Socio economic status low to moderate, n (%)	75 (27.8%)	296 (25.9%)	
Socio economic status low, n (%)	62 (23.0%)	274 (24.0%)	
Active smoking during pregnancy*, n (%)	80 (29.6%)	352 (30.8%)	0.0089
Passive smoke exposure during pregnancy*, n (%)	129 (47.8%)	479 (41.9%)	
No tobacco smoke exposure during pregnancy*, n (%)	60 (22.2%)	262 (22.9%)	
Prenatal PM_{10} exposure[#] in $\mu g/m^3$, mean ± sd	25.4 (4.7)	24.1 (4.4)	0.6574
Postnatal PM_{10} exposure[#] in $\mu g/m^3$, mean ± sd	21.3 (3.7)	22.5 (4.1)	0.6570
Functional residual capacity (FRC) at 6 weeks in ml, mean ± sd	77.7 ± 15.7	77.6 ± 16.1	0.9344
Tidal volume at 6 weeks in ml, mean ± sd	202.4 ± 43.7	197.7 ± 43.2	0.2097
Functional residual capacity (FRC) at 1 year in ml, mean ± sd	34.9 ± 6.3	34.8 ± 6.4	0.9358
Tidal volume at 1 year in ml, mean ± sd	92.5 ± 13.9	93.0 ± 14.2	0.6666
LRTI in the first year, n (%)	89 (33.0%)	359 (31.4%)	0.6627

P-values are given for the difference in study characteristics between our analysis sample and the whole study population (continuous variables tested with two-sample t-test, categorical variables i) with two categories with Fisher's exact test and ii) with more than two categories with X^2-test).

*Based on the maximum maternal prenatal cotinine level: <10 ng/ml (non-smoker), 10-499 ng/ml, (passive smoker/exposed), or ≥500 ng/ml (active smoker); LRTI: lower respiratory tract infection; NA: not available

[#]Mean difference between prenatal and postnatal PM_{10} exposure was not significant (p-value= 0.270; tested with Welch Two Sample t-test)

Table 2. Association between PM_{10} and maternal smoking exposure with lung function (FRC and tidal volume) at the age of 6 weeks and 1 year.

Time of exposure	Exposure	Lung function parameter	β-estimate (95%-CI)	p-value
Prenatal	PM_{10}	FRC (6 weeks)	-1.9 (-4.5; 0.7)	0.160
		Tidal volume (6 weeks)	-0.4 (-1.3; 0.6)	0.419
		FRC (1 year)	**-9.0 (-17.2; -0.9)**	**0.032**
		Tidal volume (1 year)	-0.2 (-2.8; 2.3)	0.851
		LRTI (in the first year)	0.0 (-0.3; 0.4)	0.915
	Maternal smoking	FRC (6 weeks)	-3.7 (-10.0; 2.6)	0.249
		Tidal volume (6 weeks)	**-2.4 (-4.7; -0.1)**	**0.043**
		FRC (1 year)	-6.7 (-25.6; 12.2)	0.487
		Tidal volume (1 year)	-4.1 (-10.1; 1.8)	0.176
		LRTI (in the first year)	**1.0 (0.2; 1.8)**	**0.016**
Postnatal	PM_{10}	FRC (1 year)	-4.3 (-12.5; 3.9)	0.304
		Tidal volume (1 year)	**-2.9 (-5.4; -0.5)**	**0.022**
		LRTI (in the first year)	0.0 (-0.3; 0.4)	0.799

LRTI: lower respiratory tract infection; effect estimates for PM_{10} are presented per increase of IQR(log(PM10+1)), resulting in 1.66 log(µg/m^3+1) for prenatal PM_{10} and 1.28 log(µg/m^3+1) for postnatal PM_{10} exposure. Effect estimates for maternal smoking are presented for active smokers with passive smoking and no tobacco smoke exposure as reference category.

All associations were adjusted for sex, birth weight (kg), age for weight z-scores at birth based on Fenton Growth Chart, maternal HIV status, ancestry of child, age and height at time of lung function measurement and socio economic status (SES) quartile. Associations with PM_{10} were additionally adjusted for maternal smoking behavior during pregnancy based on prenatal urine cotinine measures.

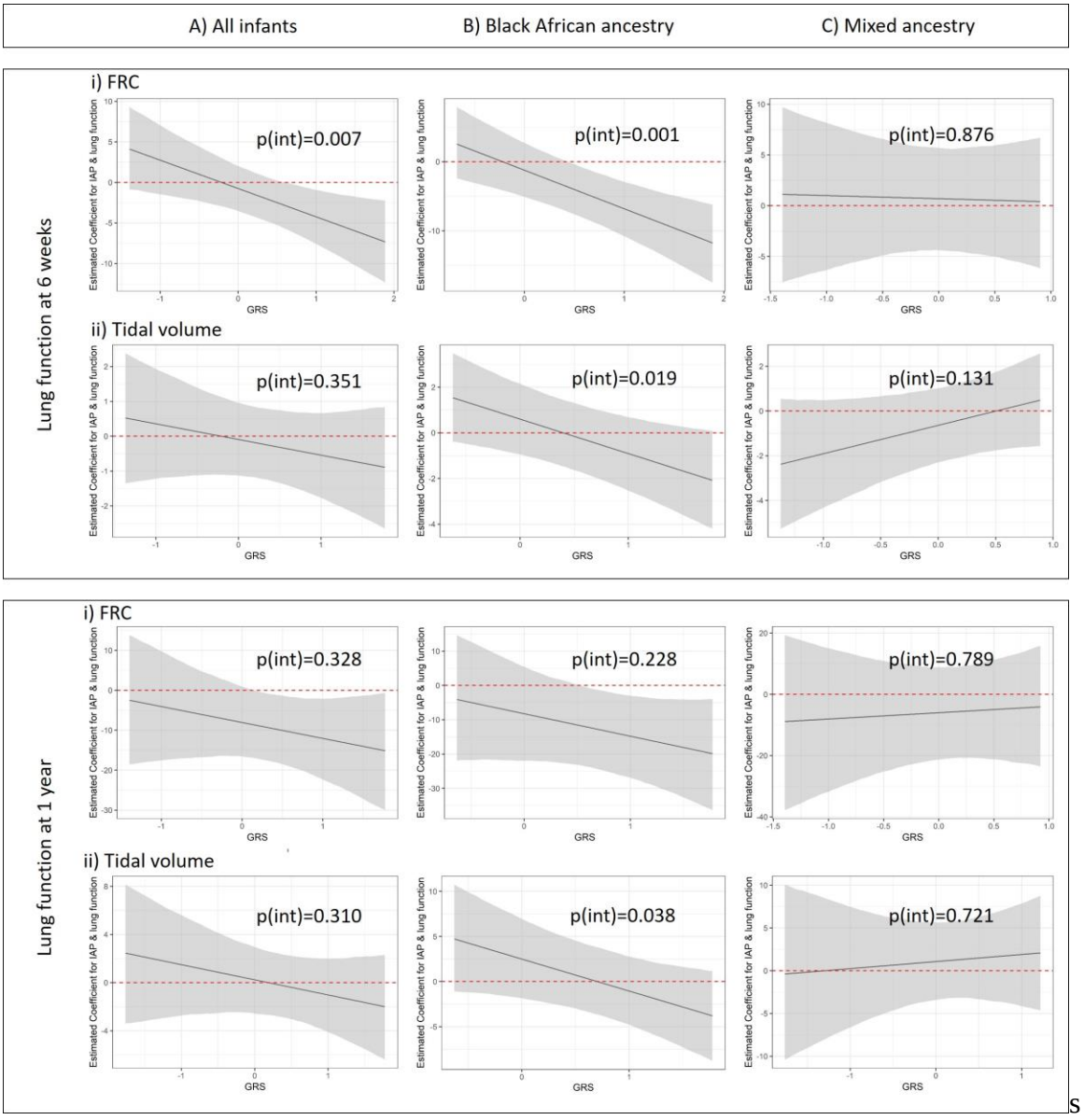
Table 3. Interaction between PRS-asthma and indoor air pollution (PM_{10} and maternal smoking exposure) on lung function (FRC and tidal volume) at the age of 6 weeks and 1 year.

			All		Black African ancestry		Mixed ancestry	
Time of exposure	**Exposure**	**Lung function parameter**	**β-estimate (95%-CI)**	**p-value**	**β-estimate (95%-CI)**	**p-value**	**β-estimate (95%-CI)**	**p-value**
Prenatal	PM_{10}	FRC (6 weeks)	**-3.5 (-6.0; -1.0)**	**0.007**	**-5.5 (-8.6; -2.4)**	**0.001**	-0.4 (-5.0; 4.3)	0.876
		Tidal volume (6 weeks)	-0.4 (-1.4; 0.5)	0.351	**-1.5 (-2.7; -0.3)**	**0.019**	1.2 (-0.4; 2.8)	0.131
		FRC (1 year)	-4.0 (-12.0; 4.0)	0.328	-6.4 (-16.7; 3.9)	0.228	2.1 (-13.3; 17.6)	0.789
		Tidal volume (1 year)	-1.3 (-3.7; 1.2)	0.310	**-3.5 (-6.7; -0.3)**	**0.038**	0.9 (-3.9; 5.6)	0.721
		LRTI (in the first year)	0.0 (-0.3; 0.4)	0.933	0.3 (-0.2; 0.8)	0.231	-0.3 (-1.0; 0.3)	0.317
	Maternal smoking	FRC (6 weeks)	3.4 (-0.9; 7.6)	0.120	6.2 (-1.0; 13.3)	0.093	-0.1 (-6.2; 6.0)	0.976
		Tidal volume (6 weeks)	0.6 (-0.9; 2.2)	0.415	0.9 (-1.7; 3.5)	0.494	1.0 (-1.0; 3.1)	0.334
		FRC (1 year)	9.0 (-3.5; 21.5)	0.159	9.3 (-10.5; 29.2)	0.359	14.3 (-3.6; 32.2)	0.123
		Tidal volume (1 year)	2.5 (-1.3; 6.3)	0.199	1.8 (-4.6; 8.2)	0.586	5.5 (-0.4; 11.4)	0.070
		LRTI (in the first year)	-0.1 (-0.6; 0.4)	0.705	-0.2 (-1.1; 0.7)	0.671	0.6 (-0.3; 1.5)	0.177
Postnatal	PM_{10}	FRC (1 year)	4.4 (-4.5; 13.3)	0.336	3.1 (-11.1; 17.3)	0.670	7.9 (-6.7; 22.4)	0.294
		Tidal volume (1 year)	-1.0 (-3.8; 1.8)	0.491	-1.7 (-6.4; 3.0)	0.487	-1.4 (-6.7; 3.8)	0.588
		LRTI (in the first year)	0.0 (-0.4; 0.4)	0.920	0.4 (-0.5; 1.4)	0.376	-0.4 (-1.2; 0.4)	0.314

LRTI: lower respiratory tract infection; effect estimates (β-coefficients), 95%-confidence intervals (95%-CI) and p-values are given for the interaction between PM_{10} or maternal smoking with the continuous GRS on lung function. PM_{10} is presented per increase of IQR(log(PM10+1)), resulting in 1.66 $\mu g/m^3$ for prenatal PM_{10} and 1.28 $\mu g/m^3$ for postnatal PM_{10} exposure. Effect estimates for maternal smoking are presented for active smokers with passive smoking and no tobacco smoke exposure as reference category. GRS are given as z-scores. All associations were adjusted for sex, birth weight (kg), age for weight z-scores at birth based on Fenton Growth Chart, maternal HIV status, ancestry of child, age and height at time of lung function measurement, socio economic status (SES) quartile, genotying array and the first five principal components to correct for population stratification (see Supplementary Figure S1). Associations with PM_{10} were additionally adjusted for maternal smoking behavior during pregnancy based on prenatal urine cotinine measures.

Figure legend

Figure 1. Interaction between GRS and prenatal PM_{10} exposure on lung function (FRC and tidal volume) at the age of 6 weeks and 1 year. Associations between PM_{10} and lung function are shown in dependence of GRS levels. P-values are shown for the interaction terms (p(int)). Effect estimates (β-coefficients) and 95%-confidence intervals (95%-CI) are presented per increase of 1.66 µg/m^3 in prenatal PM_{10} exposure (IQR(log(PM_{10}+1))). GRS are given as z-scores. All associations were adjusted for sex, birth weight (kg), age for weight z-scores at birth based on Fenton Growth Chart, maternal HIV status, ancestry of child, age and height at time of lung function measurement, socio economic status (SES) quartile, genotying array and the first five principal components to correct for population stratification (see Supplementary Figure S1). Associations with PM_{10} were additionally adjusted for maternal smoking behavior during pregnancy based on prenatal urine cotinine measures.



Supplementary Material

Genetic susceptibility to asthma increases the vulnerability to indoor air pollution

Anke Hüls PhD[1,2], Aneesa Vanker PhD[3], Diane Gray PhD[3], Nastassja Koen PhD[4,5], Julia L MacIsaac PhD[2], David TS Lin PhD[2], Katia E Ramadori BS[2], Peter D Sly PhD[6], Dan J Stein PhD[4,5], Michael S Kobor PhD[2], Heather J Zar PhD[3]

[1]Department of Human Genetics, Emory University, Atlanta, Georgia, USA

[2]Centre for Molecular Medicine and Therapeutics, BC Children's Hospital Research Institute, and Department of Medical Genetics, University of British Columbia, Vancouver, British Columbia, Canada

[3]Department of Paediatrics and Child Health, Red Cross War Memorial Children's Hospital and SA-MRC Unit on Child and Adolescent Health, University of Cape Town

[4]Department of Psychiatry and Mental Health, University of Cape Town, South Africa

[5]South African Medical Research Council (SAMRC) Unit on Risk and Resilience in Mental Disorders

[6]Children's Health and Environment Program, Child Health Research Centre, The University of Queensland, Brisbane, Queensland, Australia.

Table S3. Characteristics of GRS used for analyses. All GRS are calculated using PRSice with childhood-onset asthma (COA) in UK Biobank as reference data. The p-value threshold for which the GRS predicts the specific lung function parameter best (highest R^2) was used for analysis ("best GRS" shown here).

Ancestry	Lung function parameter	p-value threshold	R^2 GRS	#SNPs included	β-Coefficient	P-value
Black African	FRC (6 weeks)	0.05	0.01	25,978	28.99	0.155
	Tidal volume (6 weeks)	1	0.01	151,907	-36.36	0.171
	FRC (1 year)	0.3	0.03	83,340	333.44	0.106
	Tidal volume (1 year)	0.4	0.04	98,040	-118.51	0.096
	LRTI (in the first year)	0.2	0.02	65162	7.36	0.160
Mixed	FRC (6 weeks)	0.1	0.03	38,617	-85.12	0.069
	Tidal volume (6 weeks)	0.4	0.01	89,630	23.00	0.310
	FRC (1 year)	0.05	0.02	24,128	-136.91	0.203
	Tidal volume (1 year)	0.001	0.01	2,446	6.87	0.327
	LRTI (in the first year)	0.001	0.01	2446	-1.20	0.249

LRTI: lower respiratory tract infection

Table S4. Association between PM_{10} and maternal smoking exposure with lung function (FRC and tidal volume) at the age of 6 weeks and 1 year after additional adjustment for LRTI.

Time of exposure	Exposure	Lung function parameter	β-estimate (95%-CI)	p-value
Prenatal	PM_{10}	FRC (6 weeks)	-1.9 (-4.5; 0.7)	0.161
		Tidal volume (6 weeks)	-0.4 (-1.3; 0.5)	0.389
		FRC (1 year)	**-9.3 (-17.5; -1.1)**	**0.028**
		Tidal volume (1 year)	-0.2 (-2.8; 2.4)	0.882
	Maternal smoking	FRC (6 weeks)	-2.9 (-9.3; 3.5)	0.373
		Tidal volume (6 weeks)	-2.0 (-4.4; 0.3)	0.083
		FRC (1 year)	-7.7 (-26.8; 11.4)	0.431
		Tidal volume (1 year)	-4.2 (-10.2; 1.9)	0.179
Postnatal	PM_{10}	FRC (1 year)	-4.7 (-12.8; 3.4)	0.261
		Tidal volume (1 year)	**-2.9 (-5.4; -0.5)**	**0.022**

Effect estimates for PM_{10} are presented per increase of IQR(log(PM10+1)), resulting in 1.66 log(µg/m^3+1) for prenatal PM_{10} and 1.28 log(µg/m^3+1) for postnatal PM_{10} exposure. Effect estimates for maternal smoking are presented for active smokers with passive smoking and no tobacco smoke exposure as reference category.

Table S5. Association between GRS and lung function in the whole study sample.

Lung function parameter	**β-estimate (95%-CI)**	**p-value**
FRC (6 weeks)	-22.4 (-55.5; 10.7)	0.186
Tidal volume (6 weeks)	-4.7 (-22.9; 13.4)	0.608
FRC (1 year)	3.8 (-120.7; 128.2)	0.953
Tidal volume (1 year)	7.0 (-5.4; 19.4)	0.268
LRTI (in the first year)	-0.5 (-2.4; 1.4)	0.632

LRTI: lower respiratory tract infection

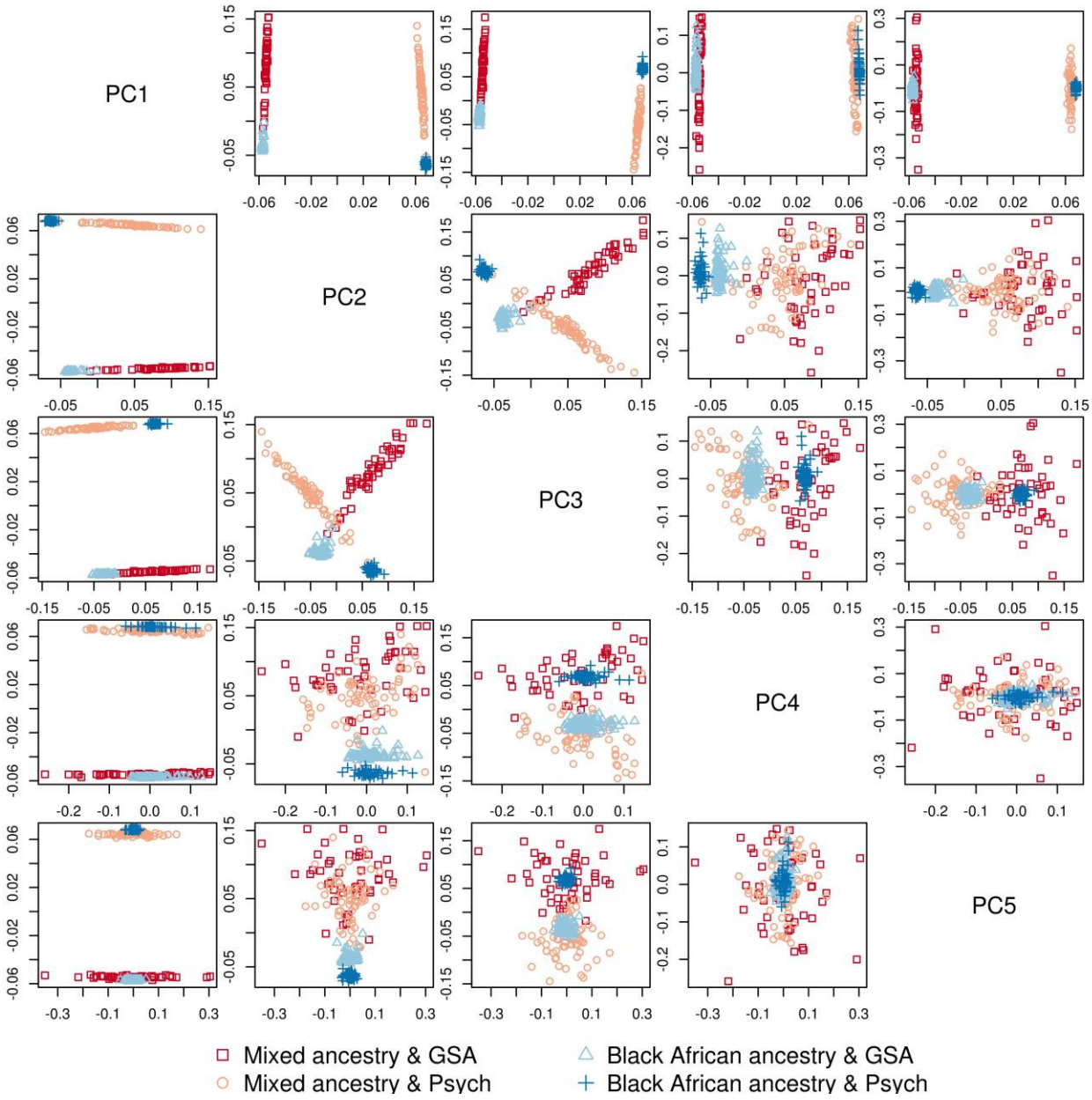


Figure S1. Correlations between the first five principal components (PC1 to PC5). Subgroups defined by ancestry and genotyping array are colored as stated in the legend.

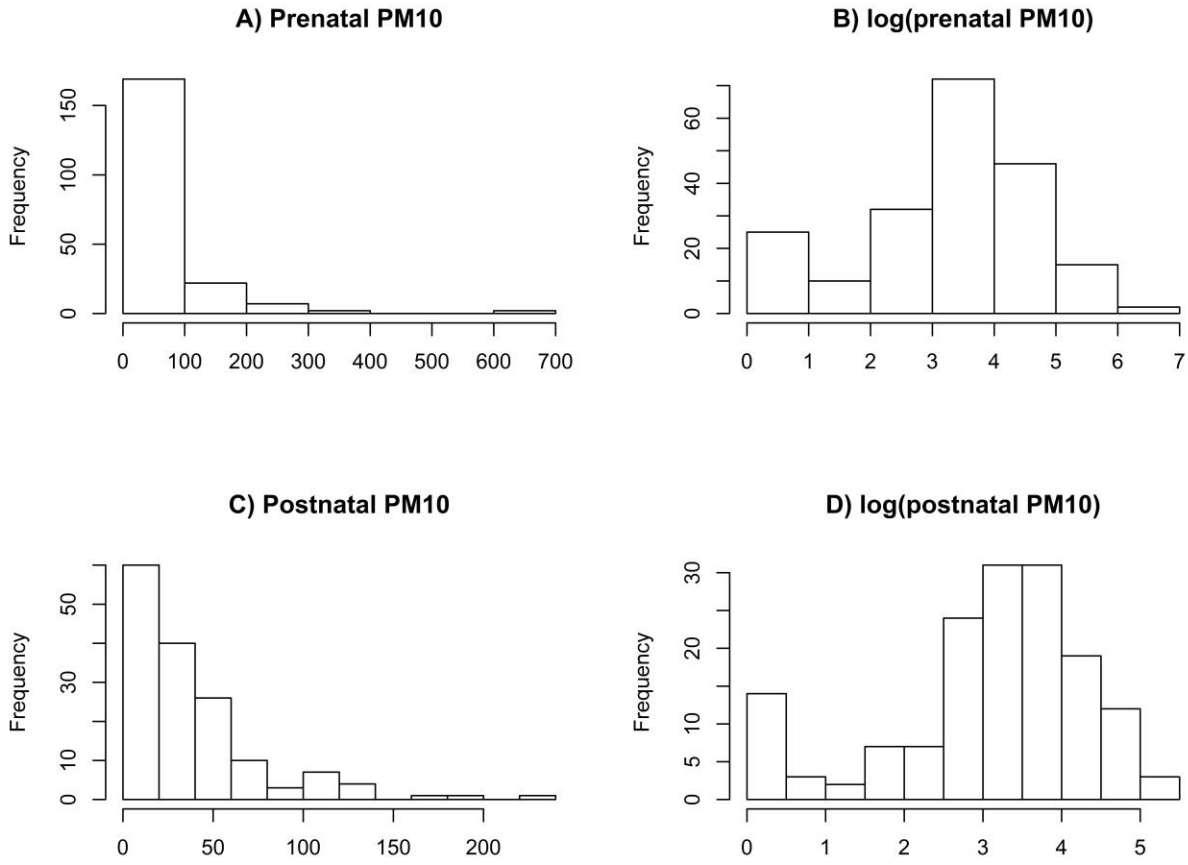


Figure S2. Distribution of prenatal and postnatal PM_{10} exposure before and after log-transformation ($\log(PM_{10} + 1)$).