Science of the Total Environment 834 (2022) 155394

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

In-utero exposure to indoor air pollution or tobacco smoke and cognitive development in a South African birth cohort study

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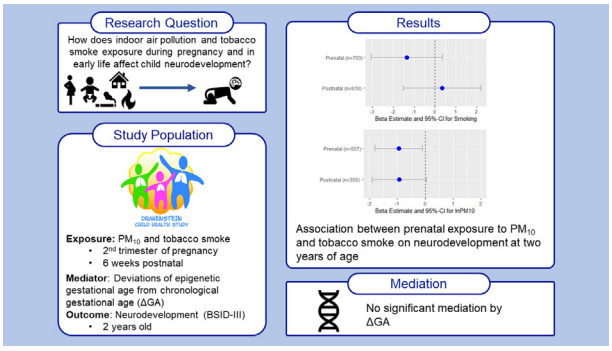
HIGHLIGHTS

- Well-characterized longitudinal birth cohort from South Africa (N = 734)
- Measured indoor air pollution data (PM_{10}) from pre- and postnatal visits
- Exposure to indoor PM_{10} was associated with poorer cognitive development.
- Investigation of epigenetic mechanisms of the PM_{10}-neurodevelopment association

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Lidia Minguez Alarcon

Keywords:
Environmental epidemiology
Epigenomics
Air pollution
Neurodevelopment
Particulate matter

ABSTRACT

Background and Aims: There is increasing evidence indicating that air pollution exposure is associated with neuronal damage. Since pregnancy is a critical window of vulnerability, air pollution exposure during this period could have adverse effects on neurodevelopment. This study aims 1) to analyze associations of prenatal exposure to indoor air pollution (particulate matter with diameters ≤10 μm, PM_{10}) and tobacco smoke with neurodevelopment and 2) to determine whether these associations are mediated by deviations of epigenetic gestational age from chronological gestational age (ΔGA).
Methods: Data of 734 children from the South African Drakenstein Child Health Study were analyzed. Prenatal PM_{10} exposure was measured using devices placed in the families' homes. Maternal smoking during pregnancy was determined by maternal urine cotinine measures. The Bayley Scales of Infant and Toddler Development III (BSID-III) was used to measure cognition, language and motor development and adaptive behavior at two years of age. Linear regression models adjusted for maternal age, gestational age, sex of child, ancestry, birth weight/length, and socioeconomic

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http://dx.doi.org/10.1016/j.scitotenv.2022.155394
Received 5 January 2022; Received in revised form 15 April 2022; Accepted 15 April 2022
Available online 20 April 2022

status were used to explore associations between air pollutants and BSID-III scores. A mediation analysis was conducted to analyze if these associations were mediated by ΔGA using DNA methylation measurements from cord blood.
Results: An increase of one interquartile range in natural-log transformed PM_{10} ($lnPM_{10}$; 1.58 μg/m^3) was significantly associated with lower composite scores in cognition, language, and adaptive behavior sub-scores (composite score β-estimate [95%-confidence interval]: −0.950 [−1.821, −0.120]). Maternal smoking was significantly associated with lower adaptive behavior scores (−3.386 [−5.632, −1.139]). Associations were not significantly mediated by ΔGA (e.g., for PM_{10} and cognition, proportion mediated [*p*-value]: 4% [0.52]).
Conclusion: We found an association of prenatal exposure to indoor air pollution (PM_{10}) and tobacco smoke on neurodevelopment at two years of age, particularly cognition, language, and adaptive behavior. Further research is needed to understand underlying biological mediators.

1. Introduction

There is increasing evidence indicating that air pollution exposure is associated with an impairment of the central nervous system (CNS) and neuronal damage. The assembly of the CNS in utero and early life requires many steps including cell proliferation, differentiation, and migration (Rodier, 2004). Failure of typical cell proliferation or cell migration due to exposure to toxic insults may have profound deleterious effects on the developing brain (Costa et al., 2004). Though neurons are able to make new synapses throughout life, the prenatal period and first few years of life are critical for the formation of the basic circuitry of the nervous system (Costa et al., 2019; Rodier, 1995). Since pregnancy and the first years of life are a critical window of developmental vulnerability for the brain (Costa et al., 2019; Rodier, 1995), few studies have investigated associations between prenatal exposure to air pollution on infants' cognitive development (Volk et al., 2021). Some studies report significant associations between both prenatal and early life exposure to PM and Autism Spectrum Disorder (ASD) in children (Lam et al., 2016; Raz et al., 2015; Volk et al., 2013). Additional studies have found prenatal particulate matter with a diameter less than 10 μm (PM_{10}) exposure to be associated with lower IQ at ages 4–6 years (Loftus et al., 2019), and decreased mental and psychomotor development scores (Kim et al., 2014).

Even fewer studies focus on indoor air pollutants, as opposed to traffic related or ambient outdoor air pollutants. Indoor air pollution is of particular concern during early life, because infants spend the majority of their time in the home (Pickett and Bell, 2011). A primary source of indoor air pollution is from combustion of gas or other fuel used to cook or heat the home (Balmes, 2019; Levy, 2015). While a few studies have shown associations between combustion of gas or other fuel inside the home and poorer mental development of the child (Vrijheid et al., 2012), developmental delay (Nazif-Muñoz et al., 2020) and delay in reaching gross motor neurodevelopmental milestones (Wei et al., 2018), none of these studies measured the actual indoor air pollution concentrations. Measuring air pollution concentrations directly is more costly than using proxy measures from questionnaires, but is important in determining what pollutant is affecting neurodevelopment. Indoor air pollution is a leading cause of death and disability worldwide (Lim et al., 2012), and is a particularly pertinent area of research for low-to-middle income countries (LMIC) due to the high percentage of households that still use traditional methods for cooking and heating (Balmes, 2019).

Another source of indoor air pollution is environmental tobacco smoke. In addition to high concentrations of indoor air pollution, smoking prevalence is also particularly high in LMICs (Islami et al., 2015). Multiple studies have reported an association between tobacco smoke exposure during pregnancy and lower child performance on measures of intelligence (Huijbregts et al., 2006; Lawlor et al., 2006), behavior problems (Roza et al., 2009), slower reaction times (Mezzacappa et al., 2011), and decreased motor abilities (Polanska et al., 2013) compared to their unexposed peers. However, all of these studies were conducted in high-income countries where exposure to tobacco smoke is typically low (Islami et al., 2015). Tobacco smoke contains PM among other pollutants and no study has tried to disentangle the effects of secondhand smoke exposure and indoor PM for neurodevelopment. In a country where exposure to tobacco smoke is more prevalent, the burden of neurodevelopmental delay associated with exposure to indoor air pollution could be greater than previously thought.

Little is known about the underlying biological pathway of how indoor air pollution affects neurodevelopment. Epigenetics has been discussed as a plausible mechanism by which environmental exposures might regulate the activity of genes relevant to child development. Epigenetic modification, specifically DNA methylation (DNAm), has been linked to a number of neuropsychiatric outcomes such as specific neurodevelopmental delays, ASD, and attention deficit hyperactivity disorder (ADHD) in several cohorts (Caramaschi et al., 2022; Mordaunt et al., 2020; Neumann et al., 2020; Walton et al., 2017) including our South African Drakenstein Child Health Study (DCHS) (Hüls et al., 2021). In addition, newborn DNAm is known to be associated with prenatal air pollution exposure as shown by studies from the Pregnancy And Childhood Epigenetics (PACE) consortium (Gruzieva et al., 2017, 2019). However, to the best of our knowledge, no study has examined the interconnections between prenatal air pollution exposure, DNAm and neurodevelopment in a mediation analysis. One challenge of epigenetic mediation analyses is the high dimension of the mediator (DNAm). One approach to address the dimensionality issue is the use of well-established risk scores that summarize information across the genome into one score (Hüls and Czamara, 2020). The most established methylation risk score is "epigenetic age", a predictor of biological age that is highly predictive of adverse health outcomes and death (Hannum et al., 2013; Horvath, 2013). Using umbilical cord blood DNAm profiles, similar estimates of epigenetic gestational age have been created (Bohlin et al., 2016; Knight et al., 2016). Similar to epigenetic age in adults, deviations of epigenetic gestational age from chronological gestational age (ΔGA) may be predictive of adverse child development and potentially mediate the association between prenatal risk factors and neurodevelopment. A previous study from Canada found indications that ΔGA might mediate the association between prenatal exposure to traffic-related air pollution and allergic sensitization in a cohort of 145 children (Sbihi et al., 2019), but it is it is currently unknown whether exposure to indoor air pollution and tobacco smoke also affects ΔGA and how ΔGA may be associated with adverse neurodevelopmental outcomes.

In this study, we aim 1) to analyze associations between pre- and postnatal exposure to indoor air pollution (PM_{10}) or tobacco smoke with neurodevelopment at 2 years of age and 2) to determine if these associations are mediated by deviations of epigenetic gestational age from chronological gestational age (ΔGA).

2. Methods

2.1. Study population and participants

The Drakenstein Child Health Study (DCHS) is a longitudinal birth cohort located in the Drakenstein sub-district of the Western Cape, South Africa, a peri-urban area 60 km outside Cape Town (Donald et al., 2018, 2019; Stein et al., 2015; Zar et al., 2015). Consenting pregnant women were enrolled at 20–28 weeks' gestation at two public primary health clinics from March 1, 2012, to March 31, 2015 (Vanker et al., 2017; Zar et al., 2015). All children were born at Paarl Hospital (Paarl, South Africa) (Vanker et al., 2017; Zar et al., 2015). Mother and infant pairs were followed after birth at 6–10 weeks old, 14 weeks old, and at 6, 9

and 12 months old and then annually thereafter (Vanker et al., 2017). Study questionnaires and clinical data were collected at enrollment and at each follow-up visit. 1137 mothers with 1143 livebirths were originally enrolled in DCHS, but only one livebirth per mother was included in this study's analysis to ensure independence among observations. The sample included in the present study were 734 children with measurements available for the cognitive Bayley Scales of Infant and Toddler Development, third edition (BSID-III) scores at two years of age.

The DCHS study was approved by the Human Research Ethics Committee of the Faculty of Health Sciences, University of Cape Town, by Stellenbosch University and the Western Cape Provincial Research committee. Written informed consent was provided by the mothers for herself and her infant and is renewed annually.

2.2. Assessment of indoor air pollution (IAP) and tobacco smoke

A prenatal (within 4 weeks of enrollment) and postnatal (between 6 and 10 weeks of the infant's life) home visit was undertaken to measure IAP; the most common pollutants and by-products of combustion were measured (Vanker et al., 2017). IAP measurements included PM_{10}, carbon monoxide (CO), nitrogen dioxide (NO_2), sulfur dioxide (SO_2), and the volatile organic compounds (VOCs) benzene and toluene. PM_{10} was collected using a personal air sampling pump (AirChek 52; SKC, Eighty Four, PA, USA), connected to a styrene filter cassette (37 mm cassette blank; SKC) with a gravimetrically pre-weighed filter (PVC filter 37 mm × 5 μm with support pad; SKC) left in the common area of the home for 24 h (Vanker et al., 2015). Filters were weighed post-sampling and analyzed using National Institute for Occupational Safety and Health method 0600 to obtain an average concentration over 24 h (Ashley, 2017). These average concentrations over 24 h were used as exposures in the analyses. Additionally, the source of heating and type of stove in each participant's house was noted. More details on home characteristics and IAP have been provided elsewhere (Vanker et al., 2015). During the postnatal home visit between 6 and 10 weeks of the infant's life, these same measurements were repeated.

Mothers also provided urine within four weeks of enrollment for analysis of cotinine levels as a measure of tobacco smoke exposure (Vanker et al., 2015). Urine cotinine levels were classified as <10 ng/ml (non-smoker), 10–499 ng/ml (passive smoker), or ≥500 ng/ml (active smoker) (Vanker et al., 2015). In addition, we used a dichotomized smoking variable indicating if the pregnant women were exposed to any (≥10 ng/ml) versus no (<10 ng/ml) tobacco smoke; these participants were categorized as exposed or non-exposed, respectively. For the postnatal assessment of tobacco smoke exposure, mothers filled out a questionnaire approximately 6–10 weeks after giving birth asking current smoking status. If the mothers indicated they were a current smoker, a secondary question asked about how many cigarettes they smoked on average per day.

2.3. Assessment of neurodevelopment

At two years of age, children in the DCHS underwent a neurodevelopment assessment using the BSID-III (Bayley, 2006). The BSID-III is one of the most frequently used directly observed tests in infant developmental assessment, and has been reported in both clinical and research settings (Albuquerque et al., 2018; Anderson and Burnett, 2017; Johnson et al., 2014; Kaya-Kara et al., 2019). Cognitive development, language development, motor development, and adaptive behavior were measured using the BSID-III. Trained assessors administered the assessment to generate scores for the cognitive, language, and motor domains via direct observation of the children, and using a caregiver-completed questionnaire for adaptive behavior (Donald et al., 2018). Composite scores were generated which have been used in previous research and validated for use in a South African setting (Ballot et al., 2017; Rademeyer and Jacklin, 2013). Additionally, for this study, an overall composite score was calculated as the average of the four domain sub-scores. If a child did not complete an assessment for each domain, the overall composite score was calculated as the average of the composite sub-scores available. Of the 1143 children originally enrolled, 734 completed at least one BSID-III domain assessment and were subsequently included in the analysis. The results of the BSID-III composite sub-scores and the overall composite score followed a normal distribution (Supplemental Fig. S1A-E).

2.4. Epigenetic age predictors

DNA was isolated from cord blood samples that were collected at time of delivery (Morin et al., 2017). DNAm data was quantified using the Illumina HumanMethylation450K BeadChip (450 K) or Infinium MethylationEPIC BeadChip (EPIC) which were subsequently combined, followed by quality control and normalization. Pre-processing and statistics were done using R 3.5.1 (R Core Team, 2018). Raw iDat files were imported to RStudio where intensity values were converted into beta values. The 450 K and EPIC datasets were then combined using the minfi package (Aryee et al., 2014) resulting in 316 samples and 453,093 probes that were available on both arrays. Background subtraction, color correction and normalization were performed using the preprocessFunnorm function (Fortin et al., 2014). After sample and probe filtering, 273 samples and 409,033 probes remained for downstream analyses (Drzymalla et al., 2021). Of these, 191 children had data available on the Bayley Scales scores at two years of age. To assess differences in PM_{10} distribution between the full BSID-III sample and this subsample, we conducted Wilcoxon Sum Rank tests.

The Bohlin-London gestational age predictor (Bohlin et al., 2016) for each participant was calculated using predictGA function (predictGA package) in R. The Bohlin-London predictor is a weighted sum of beta values of 96 CpG sites with weights as the coefficients of these CpG sites obtained from prediction models trained in 1068 cord blood DNA samples (Bohlin et al., 2016). Among the 96 CpG sites needed to compute Bohlin-London predictor, 8 were missing among EPIC array samples and 3 were missing among both 450 K and EPIC array samples. Median beta values from the 450 K array were used to impute the beta values of CpG sites missing only among the EPIC array samples and publicly available DNAm data from cord blood (GSE69176 and GSE97628) were used to impute missing values of CpG sites that were missing data from both arrays.

The Knight-Clock gestational age predictor (Knight et al., 2016) was calculated using R code available on github (https://github.com/akknight/PredictGestationalAge). This predictor is a weighted sum of beta values of 142 CpG sites, 6 of which were missing and were imputed by k-nearest neighbors method implemented in the tool.

In our study, the Bohlin-London predictor showed a higher correlation with chronological gestational age (Fig. S2AB) and was therefore used in the subsequent analyses. The Pearson correlations of the Knight-Clock and Bohlin-London predictors with chronological gestational age were 0.387 and 0.641, respectively, and there was no difference between children of Black African or mixed ancestry (Fig. S2AB).

2.5. Statistical analysis

Descriptive statistics were calculated to characterize the study population, summarizing categorical data as N (%) and continuous data as median (IQR); and Pearson correlations between all indoor air pollutants. Linear regression models were used to explore associations of indoor air pollution and maternal smoke exposure on neurodevelopment, adjusting for demographic and socioeconomic characteristics (maternal age, gestational age, sex of child, ancestry, birth weight, birth length, and prenatal parental socioeconomic status); results are presented as β-estimates and 95% confidence intervals (CI). To test if gestational age, birth weight and birth length were intermediates of the association between PM_{10}/tobacco smoke and neurodevelopment and not confounders, sensitivity models were run with and without those covariates. An additional sensitivity analysis was conducted, in which we further adjusted our association analyses with PM_{10} for season. The distribution of PM_{10} was skewed in both pre- and postnatal samples, therefore the variable was transformed using the natural log ($lnPM_{10}$) (Fig. S3). Correlations of prenatal IAPs were calculated using a

Pearson correlation and correlations of prenatal IAPs and maternal smoking were calculated using Point Biserial correlation. Results showing the association between $lnPM_{10}$ and the BSID-III overall composite score are standardized by the IQR. In main analyses, we estimated the association of prenatal PM_{10} and maternal tobacco smoke exposure with the overall composite BSID-III score as the primary outcome. Secondary analyses with each subscale composite score were conducted to assess if specific neurodevelopmental domains are more affected by indoor air pollution or tobacco smoke exposure than others. In addition to the single pollutant models, we conducted two- and multi-pollutant models. In the two-pollutant models, PM_{10} and smoking were both included in the same linear regression; which can be interpreted as the associations between PM_{10} and the BSID-III scores additionally adjusted for smoking and the associations between smoking and the BSID-III scores additionally adjusted for PM_{10}. In the multi-pollutant models, linear regression models for the associations were additionally adjusted for benzene, toluene, sulfur dioxide (SO_2), nitrogen dioxide (NO_2), and carbon monoxide (CO). To assess interaction of PM_{10} and smoking, crude, adjusted, and multi-pollutant models were run with an interaction term for PM_{10}*smoking.

To identify susceptible time windows of exposure, we analyzed associations of postnatal PM_{10} and maternal tobacco smoke exposure on the overall composite BSID-III score as well as each composite sub-score and compared the associations to the ones with the prenatal exposures. All data analysis was conducted in R (version 4.0.2).

A mediation analysis using the *mediation* R package (Tingley et al., 2014) was conducted to analyze if the associations with developmental outcomes were mediated by deviations of epigenetic gestational age from chronological gestational age (ΔGA). ΔGA was calculated as residuals that result from regressing epigenetic gestational age on chronological gestational age. Since the Bohlin-London was determined to be a more accurate predictor of gestational age, this predictor was used for the mediation analysis. A mediator model was fitted where the measure of ΔGA was modeled as a linear regression function of the exposure variable (PM_{10} or maternal tobacco smoke) while adjusting for the same variables as discussed above. Next, the outcome variable (BSID-III overall composite score, cognition subscale language subscale, motor subscale, or the adaptive behavior subscale) was modeled as a linear regression function of the mediator, exposure variable, and confounding variables as discussed above. Then the mediate function (*mediation* package) was used to estimate the average causal mediation effects (Indirect), the average direct effects (Direct), the total effects of the mediator and outcome models (Total), and the proportion of the outcome mediated by the mediator. Results of the mediation analysis determined the mediated, direct, and total effects of the exposure on the outcome with the mediated effect accounting for the influence of the mediator, (ΔGA). Sample size for mediation analyses was $n = 132$ for analyses with PM_{10} as exposure, and $n = 172$ for analyses with smoking status as the exposure, considering complete data for the exposure, mediator, and outcome.

3. Results

3.1. Description of study participants

The analysis sample included 734 children with complete information on neurodevelopment at two years of age (Table 1). Overall, about half of the children were male (51.9%). Over half of the children were of Black African ancestry (52.6%) and the rest were of mixed ancestry (47.4%). Participants were categorized into quartiles as lowest, low-to-moderate, moderate-to-high, or highest socioeconomic status based on a composite socioeconomic status score (Vanker et al., 2015; Zar et al., 2015). The percentage of participants using paraffin or fossil fuels in their stove and heating were slightly higher in the subset for multi-pollutant models compared to the full analysis sample (Table 1).

Participant demographics did not change substantially when the full cohort ($N = 1143$) was subset into those with BSID-III data ($n = 734$), those with full IAP measurements included in the multi-pollutant models ($n = 347$), and those with ΔGA ($n = 191$) (Table 1). There was low correlation between PM_{10} and the other IAPs, and low correlation between all IAPs and maternal smoking (Fig. S4).

3.2. PM_{10} and neurodevelopment

There was a significant association between an increase of one IQR (1.58 $\mu g/m^3$) in $lnPM_{10}$ and a lower overall composite BSID-III score (adjusted β-estimate [95% confidence interval]: −0.950 [−1.800, 0.1004]) (Fig. 1A, Table S1A). When maternal smoking was added in the two-pollutant model, the effect estimate for PM_{10} on BSID-III composite score increased and remained statistically significant (−1.040 [−1.8942, −0.186]). $lnPM_{10}$ was significantly associated with the cognition sub-score (−0.887 [−1.743, −0.032]), the language sub-score (−1.062 [−2.158, −0.062]), and the adaptive behavior sub-score (−1.331 [−2.464, −0.197]), but not with the motor sub-score (Fig. 2A, Table S1B). In the sensitivity analysis removing potential intermediates from the model, effect estimates were similar as in the main models. Association between PM_{10} and BSID-III composite score were robust to additional adjustment for season (Table S2–3).

When the sample was limited to only participants with full IAP measurements ($N = 347$), an IQR increase in $lnPM_{10}$ in the crude model showed a significant decrease in the BSID III overall composite score (−1.216 [−2.231, −0.200]). This association was strengthened after adjusting for confounders (−1.304 [−2.305, −0.303]). The association between $lnPM_{10}$ and the BSID-III overall composite score was robust to further adjustment of other IAPs (−1.261 [−2.269, −0.253]) (Fig. 1B, Table S1C).

3.3. Tobacco smoke and neurodevelopment

Exposure to maternal smoking was significantly associated with a lower overall composite score in the crude model (−1.672 [−3.332, −0.012]), but not after adjusting for confounding variables (−1.349 [−3.066, 0.367]). Associations were similar after adjusting for $lnPM_{10}$ exposure in the two-pollutant model ($N = 496$) (−1.818 [−3.867, 0.231]) (Fig. 1C, Table S4A). Maternal smoking during pregnancy was also significantly associated with a lower adaptive behavior scores (−3.386 [−5.632, −1.1389]), but not with the other developmental domains when adjusted for confounding variables (Fig. 2B, Table S4B). There was no significant interaction between PM_{10} and smoking for overall composite score (Table S5A).

When the sample was limited to only participants with all IAP measurements, maternal smoking was associated with the BSID-III overall composite score in the crude model (−2.404 [−4.603, −0.271]), but not after adjusting for confounders (−1.976 [−4.365, 0.414]) or other IAPs (−1.795 [−4.197, 0.642]) (Fig. 2D, Table S4C). In models with the PM_{10}*smoking interaction term, there was not a significant effect of the interaction term (Table S5B).

3.4. Identification of susceptible time window of exposure

In order to investigate if postnatal exposure to indoor air pollution or tobacco smoke was also impacting the results of the BSID-III scores, a sensitivity analysis was conducted. Due to the longitudinal and prospective nature of the study, it was possible to explore the time window of exposure at which cognitive development appears most susceptible to indoor PM_{10} and tobacco smoke. This question may be investigated by comparing linear regression models assessing the association of the BSID-III overall composite score with prenatal and postnatal exposure to the same IAP. Pre- and postnatal PM_{10} exposure was not correlated ($r = -0.003$) with each other. In contrast to prenatal exposure discussed previously, postnatal exposure to $lnPM_{10}$ (−0.935 [−1.910, 0.040]) (Fig. 3A and B, Table S6) was not associated with BSID-III composite score. Postnatal tobacco smoke (−0.351 [−1.526, 2.229]) (Fig. 3C and D, Table S7) was not associated with the BSID-III overall composite score. Postnatal $lnPM_{10}$ was associated with significantly lower cognitive score (−1.062 [−2.013, −0.110]), but not with the other domains. Postnatal tobacco smoke exposure was not

Table 1
Characteristics of birth cohort and prenatal IAP measurements for sample population subgroups.

	Full cohort	Final analysis sample	Subset for multi-pollutant models	Subset for mediation analyses with ΔGA
N	1143	734	347	191
Maternal Age (yrs) (median [IQR])	26.07 [22.29, 31.09]	26.59 [22.49, 31.35]	26.96 [22.39, 27.47]	26.61 [22.50, 31.96]
Infant Sex = Male (%)	589 (51.5)	379 (51.6)	191 (55.04)	109 (57.07)
Gestational Age (wks) (median [IQR])	39.00 [37.00, 40.00]	39.00 [38.00, 40.00]	39.00 [38.00, 40.00]	39.00 [38.00, 40.00]
Birth Weight (kg) (median [IQR])	3.09 [2.71, 3.42]	3.10 [2.71, 3.41]	3.12 [2.82, 3.44]	3.12 [2.76, 3.44]
Birth Length (cm) (median [IQR])	50.00 [48.00, 52.00]	50.00 [48.00, 52.00]	51.00 [49.00, 53.00]	51.00 [48.00, 58.00]
Ancestry				
Mixed ancestry (%)	511 (44.7)	348 (47.4)	167 (48.13)	92 (48.17)
Black African ancestry (%)	632 (55.29)	386 (52.59)	180 (51.87)	99 (51.83)
Parental Socioeconomic status (%)				
Lowest SES	274 (24.16)	180 (24.5)	89 (25.65)	48 (25.13)
Low-moderate SES	296 (26.1)	196 (26.7)	94 (27.09)	52 (27.23)
Moderate-high SES	290 (25.57)	193 (26.3)	94 (27.09)	49 (25.65)
Highest SES	283 (24.96)	165 (22.5)	70 (20.17)	42 (21.99)
Paraffin Stove (%)	128 (17.44)	128 (17.44)	76 (21.90)	21 (10.99)
Fossil Stove (%)	176 (23.98)	176 (23.98)	107 (30.84)	28 (14.66)
Paraffin Heating (%)	129 (17.57)	129 (17.57)	77 (22.19)	20 (10.47)
Fossil Heating (%)	141 (19.21)	141 (19.21)	87 (25.07)	21 (10.99)
Prenatal Maternal Smoking = Exposed (%)	831 (76.03)	559 (78.1)	261 (75.22)	147 (76.96)
Prenatal maternal smoking (%)				
Non-Smoker	262 (23.99)	157 (21.90)	86 (24.78)	44 (23.04)
Passive Smoker	479 (43.86)	308 (43.00)	143 (41.21)	83 (43.46)
Active Smoker	352 (32.23)	251 (35.10)	118 (34.01)	64 (33.51)
Postnatal Maternal Smoking = Exposed (%)	252 (27.97)	187 (30.11)	91 (30.23)	49 (28.65)
Prenatal Benzene Concentration ($\mu g/m^3$) (median [IQR])	4.28 [1.75, 11.29]	3.67 [1.45, 9.71]	4.28 [1.51, 10.48]	4.34 [2.41, 9.52]
Prenatal Toluene Concentration ($\mu g/m^3$) (median [IQR])	16.79 [7.04, 44.24]	16.57 [6.79, 46.24]	17.63 [8.12, 52.67]	17.63 [8.79, 46.50]
Prenatal SO_2 Concentration ($\mu g/m^3$) (median [IQR])	0.00 [0.00, 0.28]	0.00 [0.00, 0.28]	0.00 [0.00, 0.34]	0.00 [0.00, 0.29]
Prenatal NO_2 Concentration ($\mu g/m^3$) (median [IQR])	7.13 [3.33, 12.69]	6.61 [3.36, 12.59]	6.92 [3.49, 10.57]	8.58 [4.59, 13.50]
Prenatal PM_{10} Concentration ($\mu g/m^3$) (median [IQR])	33.37 [12.49, 64.80]	35.51 [13.17, 67.57]	33.45 [14.63, 64.41]	38.89 [14.53, 73.88]
Prenatal CO Concentration (mg/m^3) (median [IQR])	0.00 [0.00, 6.02]	0.00 [0.00, 2.86]	0.00 [0.00, 7.88]	0.00 [0.00, 6.58]
Postnatal Benzene Concentration ($\mu g/m^3$) (median [IQR])	3.08 [1.06, 9.46]	2.61 [0.90, 7.34]	3.23 [0.83, 11.05]	3.69 [1.30, 14.08]
Postnatal Toluene Concentration ($\mu g/m^3$) (median [IQR])	15.50 [5.90, 48.97]	13.26 [5.42, 47.15]	17.04 [6.66, 63.73]	18.68 [6.14, 52.98]
Postnatal SO_2 Concentration ($\mu g/m^3$) (median [IQR])	0.00 [0.00, 0.00]	0.00 [0.00, 0.00]	0.00 [0.00, 0.00]	0.00 [0.00, 0.00]
Postnatal NO_2 Concentration ($\mu g/m^3$) (median [IQR])	5.83 [2.58, 12.55]	5.28 [2.57, 11.81]	6.15 [2.57, 12.24]	6.36 [2.59, 13.90]
Postnatal PM_{10} Concentration ($\mu g/m^3$) (median [IQR])	29.47 [12.66, 52.47]	29.28 [12.48, 53.13]	25.86 [9.86, 49.17]	24.38 [12.41, 48.69]
Postnatal CO Concentration (mg/m^3) (median [IQR])[a]	0.00 [0.00, 0.00]	0.00 [0.00, 2.09]	0.00 [0.00, 4.65]	0.00 [0.00, 15.94]
Bayley Scales-Cognitive (median [IQR])	85.00 [80.00, 90.00]	85.00 [80.00, 90.00]	85.00 [80.00, 90.00]	85.00 [80.00, 90.00]
Bayley Scales-Language (median [IQR])	83.00 [77.00, 91.00]	83.00 [77.00, 91.00]	83.00 [77.0, 93.25]	85.00 [74.00, 91.00]
Bayley Scales-Motor (median [IQR])	92.50 [85.00, 100.00]	92.50 [85.00, 100.00]	94.00 [85.00, 100.00]	91.00 [85.00, 100.00]
Bayley Scales-Adaptive Behavior (median [IQR])	84.00 [72.25, 92.00]	84.00 [72.25, 92.00]	84.00 [74.99, 92.00]	84.00 [71.00, 92.00]
Bayley Scales-Overall Composite (median [IQR])	86.25 [80.25, 92.25]	86.25 [80.25, 92.25]	87.33 [81.83, 93.67]	86.67 [81.33, 93.67]

[a] A majority of participants (76%) have a postnatal CO measurement of 0 mg/m^3.

significantly associated with performance in any of the developmental domains.

3.5. Mediation by ΔGA

Distribution of PM_{10} was not significantly different in this subsample compared to the full BSID-III sample (Table S8). Our subsequent mediation analysis did not support the role of ΔGA as mediating pathway for the association between indoor air pollution or smoking and neurodevelopment. Only 1% [p-value = 0.95], 4% [0.52], 4% [0.74], −5% [0.70] of the association between PM_{10} and the overall composite score, cognition, language, and motor function were mediated by ΔGA, respectively (Fig. 4A-D). While 20% [0.13] of the association between PM_{10} and adaptative behavior was mediated by ΔGA, this finding needs to be interpreted with caution due to the lack of a significant total effect (Fig. 4E). Similarly, only −4% [0.79], 2% [0.92], 2% [0.87], −4% [0.92], 6% [0.52] of the association between maternal smoking and the overall composite score, cognition, language, motor, and adaptive behavior were mediated by ΔGA, respectively (Fig. 4F-J).

4. Discussion

In this prospective longitudinal cohort study of pregnant women in South Africa whose children underwent a neurodevelopmental assessment at two years of age, our findings suggest that in-utero exposure to indoor air pollution and tobacco smoke may be negatively associated with neurodevelopment at two years of age. Exposure to indoor PM_{10} during pregnancy was associated with poorer developmental outcomes particularly for cognitive and language domains, as well as for adaptive behavior Even after adjusting for confounding variables, gestational age, smoking and other IAPs (CO, NO_2, SO_2, VOCs), PM_{10} remained significantly associated with the lower overall BSID-III composite score. The increased effect estimate in the full IAP subsample may be due to uncertainty in the model, as wider 95% CIs are observed. Though, the significant association indicates that PM_{10} is an important neurotoxicant independent of other IAPs. There were no significant interaction between PM_{10} and smoking in any models assessing effect on BSID-III overall composite score. The association between PM_{10} and neurodevelopment was only significant for prenatal exposure (not postnatal exposure), indicating that pregnancy is a critical period for neurodevelopment and vulnerability to IAP. Postnatal PM_{10} was significantly associated with the cognitive domain performance, the postnatal early life period may be a sensitive window for PM_{10} exposure. We did not find any indication that ΔGA may be a mediating pathway for the association between IAP and BSID-III scores. Further studies are needed to better understand the biological mechanisms of how exposure to air pollutants causes neurological changes and the role of epigenetics.

Our study addresses an important gap in the current literature on air pollution and neurodevelopment by providing the first evidence for an

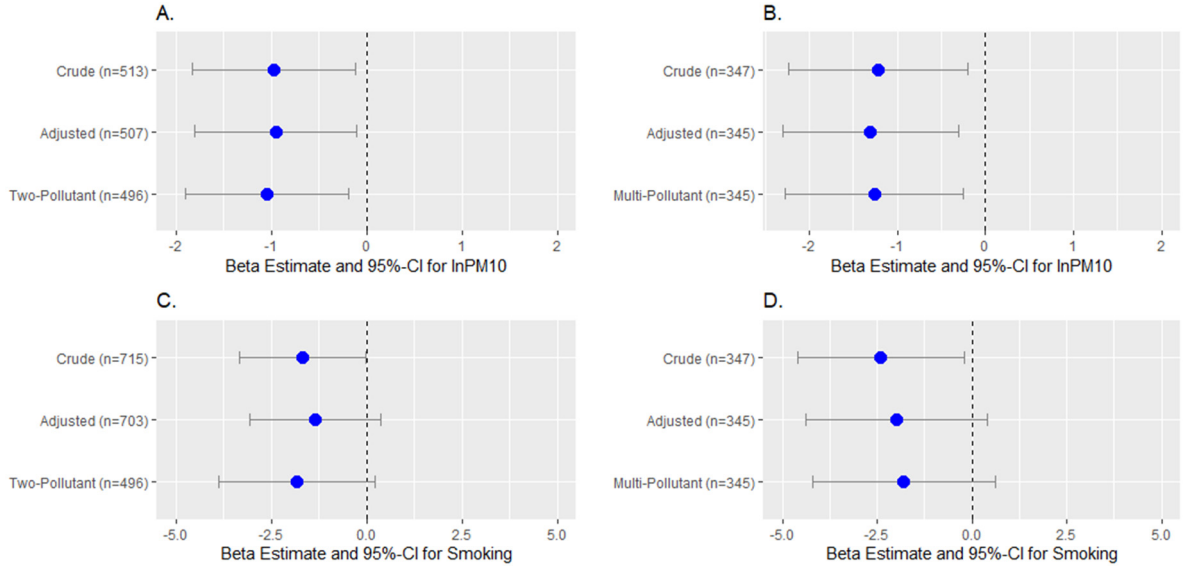

Fig. 1. Association of $lnPM_{10}$ and smoking on Bayley Scales overall composite score. A. $lnPM_{10}$ and Bayley Scales overall composite score (single and two-pollutant models, standardized by IQR of $lnPM_{10}$ [1.58 $\mu g/m^3$]). Crude: crude association between $lnPM_{10}$ and Bayley score; Adjusted: adjusted for maternal age, gestational age, sex of child, ancestry, birth weight, birth length, and parental socioeconomic status; Two-pollutant: additionally adjusted for maternal smoking. B. $lnPM_{10}$ and Bayley Scales overall composite score (single and multi-pollutant models, standardized by IQR of $lnPM_{10}$ [1.58 $\mu g/m^3$]). Crude: crude association between PM_{10} and Bayley score in the subsample, in which all IAPs (PM_{10}, benzene, toluene, SO_2, NO_2, CO) were available; Adjusted: adjusted for maternal age, gestational age, sex of child, ancestry, birth weight, birth length, and parental socioeconomic status; Multi-Pollutant: additionally, adjusted for maternal smoking and all other IAPs. C. Smoking and Bayley Scales overall composite score (single and two-pollutant models). Crude: crude association between maternal tobacco smoke and Bayley score; Adjusted: adjusted for maternal age, gestational age, sex of child, ancestry, birth weight, birth length, and parental socioeconomic status; Two-pollutant: additionally adjusted for PM_{10}. D. Smoking and Bayley Scales overall composite score (single and multi-pollutant models). Crude: crude association between smoking and Bayley score in the subsample, in which all IAPs were available; Adjusted: additionally, adjusted for maternal age, gestational age, sex of child, ancestry, birth weight, birth length, and parental socioeconomic status; Multi-Pollutant: additionally, adjusted for PM_{10} and IAPs.

association between measured indoor PM_{10} concentrations and neurodevelopmental outcomes in very young children. While no other studies researched the specific effect of prenatal PM_{10} exposure on neurodevelopment using BSID-III, PM_{10} is thought to be neurotoxic and has been associated with reduced IQ, poorer mental and psychomotor development, and ASD diagnosis (Costa et al., 2019; Kim et al., 2014; Loftus et al., 2019; Volk et al., 2013). In addition, one study found outdoor $PM_{2.5}$ exposure during pregnancy was associated with lower motor scores in the BSID (Lertxundi et al., 2015). Lertxundi et al. did not measure PM_{10} exposure, but PM_{10} and $PM_{2.5}$ are typically highly correlated (Eeftens et al., 2012). Conversely, a limitation of our study is that $PM_{2.5}$ was not measured, this highlights the need for broad pollutant monitoring in epidemiology studies, Indoor PM_{10} can come from a variety of sources including smoking, cooking using kerosene and biomass fuels, woodstoves

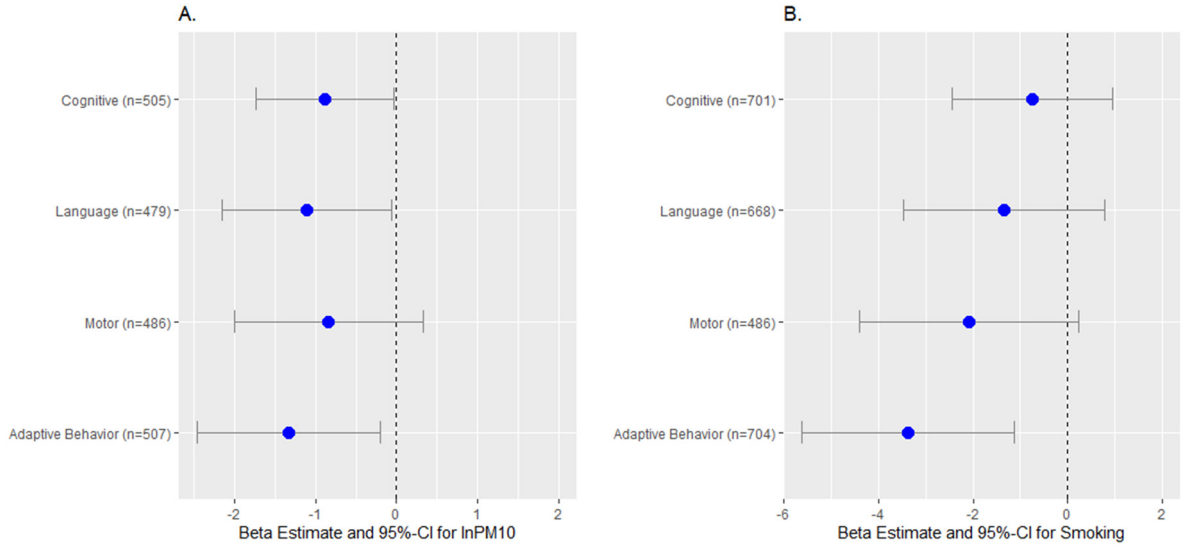

Fig. 2. Association of $lnPM_{10}$ and smoking on Bayley Scales sub-scores. A. Single pollutant analysis of $lnPM_{10}$ exposure (per IQR of PM_{10} [1.58 $\mu g/m^3$]) on the Bayley Scales four composite sub-scores adjusted for maternal age, gestational age, sex of child, ancestry, birth weight, birth length, and parental socioeconomic status. B. Single pollutant analysis of maternal tobacco smoke exposure on the Bayley Scales four composite sub-scores adjusted for maternal age, gestational age, sex of child, ancestry, birth weight, birth length, and parental socioeconomic status.

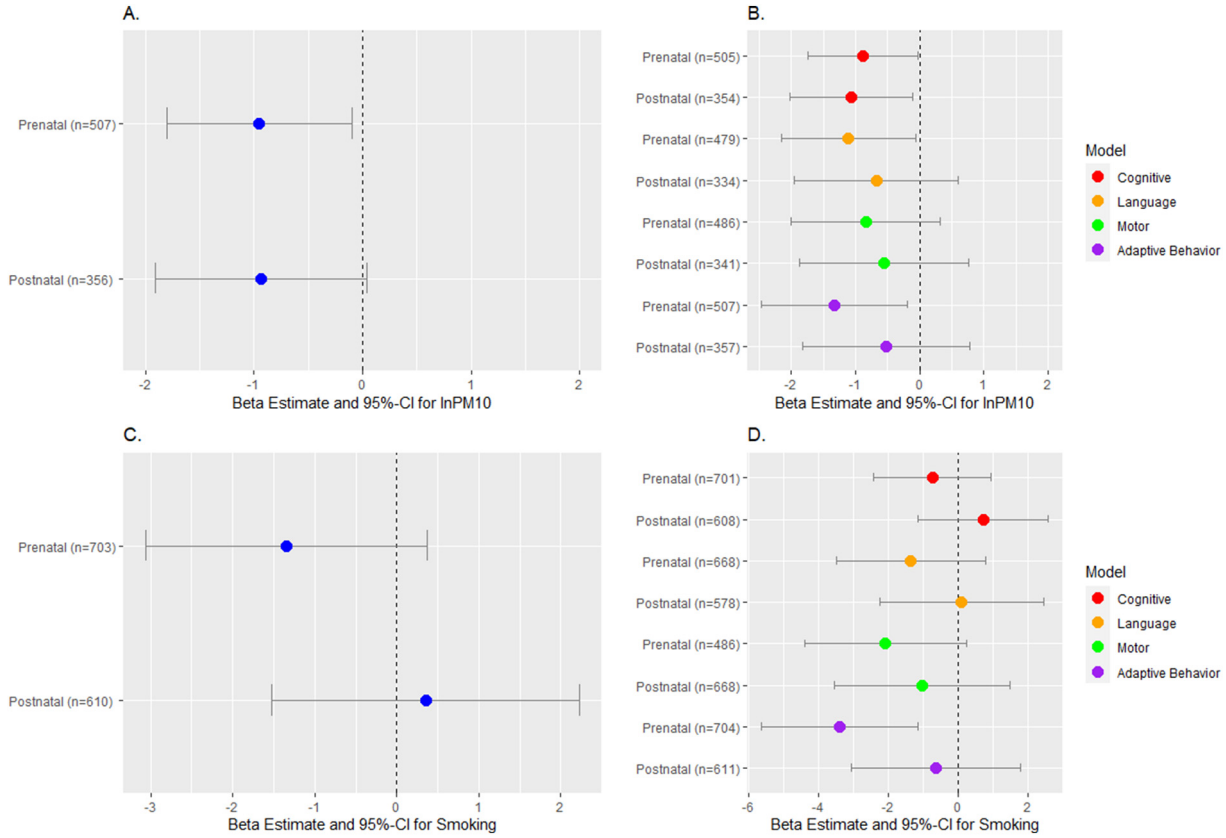

Fig. 3. Identification of the most vulnerable time window of exposure. A. $lnPM_{10}$ (per IQR) and Bayley Scales overall composite score. B. $lnPM_{10}$ (per IQR) and Bayley Scales composite sub-scores. C. Smoking and Bayley Scales overall composite score. D. Smoking and Bayley Scales composite sub-scores. Prenatal: Prenatal $lnPM_{10}$ or tobacco smoke exposure (within 4 weeks of enrollment), Postnatal: Postnatal $lnPM_{10}$ or tobacco smoke exposure (between 6 and 10 weeks of the infant's life); IQR prenatal $lnPM_{10}$ exposure: 1.58 $\mu g/m^3$; IQR postnatal $lnPM_{10}$ exposure: 1.39 $\mu g/m^3$. All associations were adjusted for maternal age, gestational age, sex of child, ancestry, birth weight, birth length, and parental socioeconomic status.

and furnaces (Vardoulakis et al., 2020). These sources, as opposed to sources of outdoor pollutants, can be easily modified by individuals to reduce PM_{10} concentrations and exposure in the home. Identifying specific pollutants that affect neurodevelopment is important for identifying and eliminating the source of those pollutants to prevent exposure and subsequent health outcomes. Future studies can incorporate outdoor exposure measurements for a holistic assessment of air pollution exposure.

Exposure to maternal tobacco smoke was not associated with lower overall neurodevelopment in adjusted models, except with respect to BSID-III adaptive behavior outcomes. These findings are in line with previous studies that have found self-reported environmental tobacco smoke exposure during pregnancy to be associated with specific domains of development as measured on the BSID-III, specifically cognition and language (He et al., 2018) (B.-E. Lee et al., 2011; M. Lee et al., 2019). Another study that also used maternal urinary cotinine levels to assess maternal tobacco smoke exposure found cotinine levels were associated with mental development index scores, in a study using the Korean version of the Bayley Scales of Infant Development version II (K-BSID-II) (B.-E. Lee et al., 2011; M. Lee et al., 2019).

The two-pollutant models that included both PM_{10} and smoking status reached statistical significance for PM_{10} and not smoking. Though, effect estimates did not differ between models that were or were not adjusted for co-exposure to PM_{10} and smoking. This suggests that there may be independent effects of PM_{10} and smoking on neurodevelopment. While smoking cigarettes does emit PM_{10} into the home, cigarettes also expose the smoker to other toxicants that may harm the developing fetus (Avino et al., 2018).

Subsequently, our findings indicated that the observed significance of indoor air pollution exposure on neurodevelopment is primarily the result of the prenatal, in-utero exposure, rather than postnatal early-life exposure. Previous studies have reported that the second trimester was the most sensitive time window for behavioral and active muscle tone development (Chen et al., 2020), indicating that this trimester could also be highly sensitive to air pollution exposure. However, this study only investigated exposure during the second trimester, so future studies would be needed to assess sensitive windows in other stages of pregnancy. In contrast to the present study, other studies have found both pre- and postnatal secondhand smoke exposure associated with poorer neurodevelopmental outcomes at two years (Mohamed et al., 2018). This may, however, be due to the use of different outcome measures of neurodevelopment, the use of self-reported smoking status in the postnatal period as opposed to measured cotinine levels, or due to limited statistical power of our analyses. A limitation of the sensitivity analyses comparing pre- and postnatal exposure periods is the differential measurement of exposure to tobacco smoke. These differences could potentially explain stronger associations with prenatal tobacco smoke exposure than with postnatal exposures.

In our study, the association of prenatal exposure to PM_{10} and tobacco smoke on neurodevelopment were not significantly mediated by epigenetic gestational age (measured by DNAm in cord blood). So far, only one study has investigated ΔGA as a potential mediating pathway for the adverse effects of prenatal air pollution exposure (Sbihi et al., 2019). Sbihi et al. found indications that ΔGA might mediate the association between prenatal exposure to traffic-related air pollution and allergic sensitization in a cohort of 145 children (Sbihi et al., 2019). Future studies should investigate the role of other epigenetic markers in addition to ΔGA as potential mediators for the association between air pollution and neurodevelopment using high-dimensional mediation

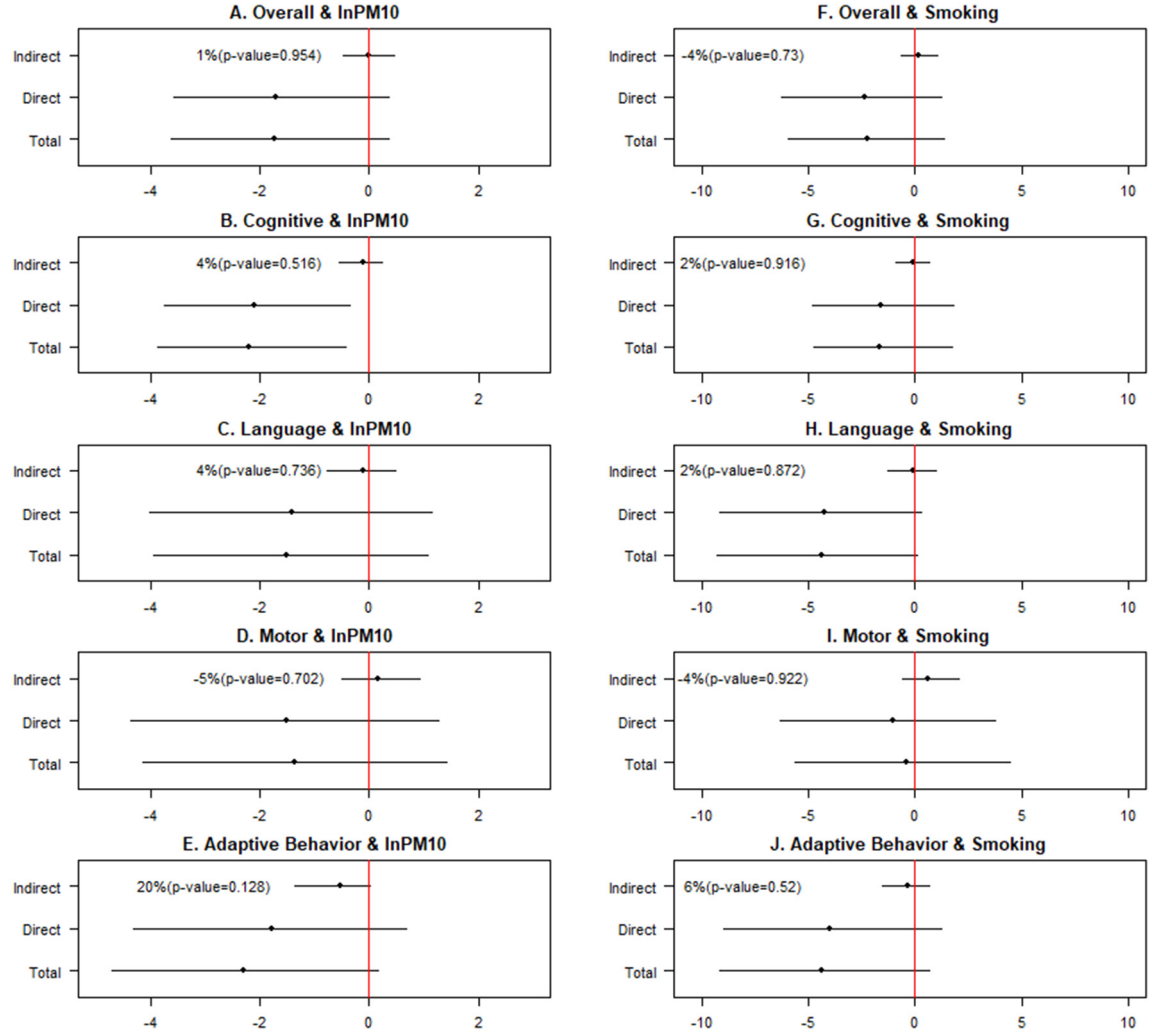

Fig. 4. Mediation analysis for deviations of epigenetic gestational age from chronological gestational age (ΔGA). A mediation analysis was conducted to analyze if the associations of $lnPM_{10}$ and smoking on the Bayley scores were mediated by ΔGA. All figures show the average causal mediation effects (Indirect), the average direct effects (Direct), the total effects of the mediator and outcome models (Total), and the proportion of the outcome mediated by the mediator (in % with *p*-value). A. Mediation analysis of ΔGA (mediator) and $lnPM_{10}$ exposure on Bayley Scales overall composite score B-E. Mediation analysis of ΔGA (mediator) and $lnPM_{10}$ exposure on the four Bayley Scales sub-scores. F. Mediation analysis of ΔGA and maternal tobacco smoke exposure on Bayley Scales overall composite score. G-J. Mediation analysis of ΔGA and maternal tobacco smoke exposure on the four Bayley Scales sub-scores. All models were adjusted for maternal age, gestational age, sex of child, ancestry, birth weight, birth length, and parental socioeconomic status.

analysis or pre-selected CpG sites based on their known association with air pollution and/or neurodevelopment.

The DCHS is a unique birth cohort that enables the analysis of various exposure assessments over time to investigate potential associations between in-utero air pollution and neurodevelopment due to prenatal recruitment and postnatal follow-ups. Prenatal recruitment allows assessment of exposure during gestation and after birth, use of a population-based sample helps to eliminate selection biases inherent in case-based approaches, and the cohort design ensures clear identification of the time-order of associations (Zar et al., 2015). Additional strengths include a large sample size that evaluated high-risk infants using the BSID-III, a well- established assessment tool. Though, not all children in the cohort completed the BSID-III, limiting sample size in this study (Wedderburn et al., 2019). The limitations of this study include the short PM_{10} exposure record time, and missing values for IAPs and DNAm. Although PM_{10} was recorded prenatally and postnatally and therefore, highlighted multiple time points, it was only recorded for 24 h at each assessment. Therefore, this measurement could potentially incorrectly reflect actual indoor air pollution exposure. This may also explain low correlation between pre- and postnatal PM_{10} measurements. Additionally, IAP measurements were only available for a subsample, reducing the sample size in multi pollutant models. There was also reduced sample size for analyses using DNAm, because only a subsample of participants had DNAm data. Another limitation was the one-time measurement of the neurodevelopment assessment. While neurodevelopment occurs over multiple years, the focus of this study was to examine the impact of IAP on early cognitive development. Future studies should look at the association between prenatal exposure to air pollution and the trajectory of neurodevelopmental outcomes over the early years of life. Additionally, future studies can introduce interventions aimed at preventing IAP exposure to reduce neurodevelopmental outcomes.

To our knowledge, this is the first study to assess prenatal exposure to measured indoor air pollutants on neurodevelopment in a large cohort study, and therefore, addresses an important gap in epidemiologic and environmental health research. Moreover, this is also the first study to include research on the epigenetic mechanisms that may be influencing neurodevelopment in the context of exposure to indoor air pollutants. Although our findings determined these associations are not significantly mediated by ΔGA, other epigenetic mechanisms could be influencing neurodevelopment upon in-utero exposure to air pollution. Additionally,

our study supports the view that pregnancy is a vulnerable time window of exposure for neurodevelopment, emphasizing the importance of pregnancy as a time to implement integrated approaches to support healthy lifestyles and reduce substance use and other factors associated with neurodevelopmental risk in the unborn child.

5. Conclusion

In summary, our findings show a significant association between prenatal exposure to indoor air pollution (PM_{10}) and tobacco smoke on neurodevelopment at two years of age. Further research to understand underlying biological mediators of toxic exposure and neurodevelopment is needed.

CRediT authorship contribution statement

Grace M. Christensen: Formal analysis - data analysis and visualization; Writing - original draft preparation, Claire Rowcliffe: Formal analysis - data analysis and visualization; Writing - original draft preparation, Junyu Chen: Formal analysis – calculation of epigenetic predictors for gestational age; Writing - Review & Editing, Aneesa Vanker: Investigation and Data Curation – assessment of indoor air pollution and tobacco smoke exposure; Writing - Review & Editing, Nastassja Koen: Investigation and Data Curation; Writing - Review & Editing, Meaghan J. Jones: Writing - Review & Editing, Nicole Gladish: Data Curation – quality control and preprocessing of DNA methylation data; Writing - Review & Editing, Nadia Hoffman: Project administration, Kirsten A. Donald: Investigation and Data Curation; Writing - Review & Editing, Catherine J. Wedderburn: Writing - Review & Editing; Michael S Kobor: Writing - Review & Editing, Supervision – generation of DNA methylation data, Heather J Zar: Supervision and Funding acquisition; Writing - Review & Editing, Dan J Stein: Supervision and Funding acquisition; Writing - Review & Editing, Anke Hüls: Conceptualization, Methodology, Writing - original draft, Supervision.

Funding

The Drakenstein Child Health Study was funded by the Bill & Melinda Gates Foundation (OPP 1017641), Discovery Foundation, South African Medical Research Council, National Research Foundation South Africa, CIDRI Clinical Fellowship and Wellcome Trust (204755/2/16/z). Additional support for the DNA methylation work was provided by the Eunice Kennedy Shriver National Institute of Child Health and Human Development of the National Institutes of Health (NICHD) under Award Number R21HD085849, and the Fogarty International Center (FIC). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. AH is supported the HERCULES Center (NIEHS P30ES019776). GMC is supported by the NIEHS T32 Training Program in Environmental Health and Toxicology (5T32ES12870). CJW was supported by a Wellcome Trust fellowship (203525/Z/16/Z). DJS and HJZ are supported by the South African Medical Research Council (SAMRC). The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.155394.

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