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Associations between pre- and postnatal exposure to air pollution and lung health in children and assessment of CC16 as a potential mediator

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ABSTRACT

Background: Early life exposure to air pollution can affect lung health. Previous studies have not assessed the implications of both pre- and postnatal exposure to air pollutants on lung function at repeated ages during childhood. In addition, there is the need to identify potential mediators of such effect.
Objectives: To longitudinally assess the association between pre- and postnatal air pollution exposure and lung function during childhood. We also aimed to explore the role of Club cell secretory protein (CC16) as a potential mediator in this association.
Methodology: We included 487 mother-child pairs from the INMA (INfancia y Medio Ambiente) Sabadell birth cohort, recruited between 2004 and 2006. Air pollution exposure was estimated for pregnancy, pre-school age, and school-age using temporally adjusted land use regression (LUR) modelling. Lung function was measured at ages 4, 7, 9 and 11 by spirometry. At age 4, serum CC16 levels were determined in 287 children. Multivariable linear regression models and linear mixed modelling were applied, while considering potential confounders.
Results: Prenatal exposure to Particulate Matter $(PM)_{10}$ and PM_{coarse} had the most consistent associations with reduced lung function in cross-sectional models. Associations with postnatal exposure were less consistent. Increasing CC16 levels at 4 years were associated with an increase in FEF_{25-75} ($\beta = 120.4$ mL, 95% CI: 6.30, 234.5) from 4 to 11 years of age. No statistically significant associations were found between pre- or postnatal air pollution and CC16 at age 4.
Conclusion: Increasing levels of air pollution exposure, particularly prenatal PM_{10} and PM_{coarse} exposure, were associated with a reduction in lung function. We were not able to confirm our hypothesis on the mediation role of CC16 in this association, however our results encourage further exploration of this possibility in future studies.

1. Introduction

The developing lung during pregnancy and early life is particularly susceptible to damage from environmental exposures (Miller and Marty, 2010; Stocks et al., 2013). Children tend to have a faster breathing rate than adults, which increases the circulation of particles within the lungs, and thus increases the dose of these particles absorbed in children (Saadeh and Klaunig, 2014). In addition, children usually spend more time outside than adults, which exposes them to greater levels of air pollution (Goldizen et al., 2016; Schwartz, 2004). Individuals who have diminished lung function in their childhood are at increased risk of diminished lung function in early adulthood and of developing serious

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diseases such as asthma and chronic obstructive pulmonary disease (COPD) within their lifetime (Carraro et al., 2014; Stocks et al., 2013).

Increased levels of pre- and postnatal exposure to air pollutants [e.g. nitrogen dioxide (NO_2), carbon monoxide (CO), ozone (O_3), benzene, particulate matter (PM) - including ultrafine particles (UFP), $PM_{2.5}$, PM_{10}, PM_{coarse}, sulphur dioxide (SO_2), or polycyclic aromatic hydrocarbons (PAHs)] have been related to adverse respiratory health outcomes in children, including increased risk of respiratory infections (Dherani et al., 2008; Jedrychowski et al., 2015; MacIntyre et al., 2014; Mehta et al., 2013); increased incidence and prevalence of asthma (Clark et al., 2010; Deng et al., 2016; Hsu et al., 2015; Shima et al., 2002); and increased number of hospital admissions due to respiratory illness (Barnett et al., 2005; Koranteng et al., 2007; Preutthipan et al., 2004). Several studies have examined the effects of early life air pollution exposure on lung function, with most showing a decrease in lung function with increasing exposure to air pollution. These studies have mainly focused on either prenatal (Bose et al., 2018; Jedrychowski et al., 2010; Latzin et al., 2009; Lee et al., 2018; Mortimer et al., 2008) or postnatal exposure (Barone-Adesi et al., 2015; Bergstra et al., 2018; Frye et al., 2003; Fuertes et al., 2015; Gauderman et al, 2004, 2015; Gehring et al., 2013; Hwang et al., 2015; Milanzi et al., 2018; Mölter et al., 2013; Oftedal et al., 2008; Rojas-Martinez et al., 2007; Schultz et al, 2012, 2016; Urman et al., 2014; Usemann et al., 2019) to air pollution. Fewer have examined the effects of both pre- and postnatal air pollution exposure on lung function (Bougas et al., 2018; Cai et al., 2020; Jedrychowski et al., 2015; Korten et al., 2017; Morales et al., 2015; Mortimer et al., 2008), with each study reporting reduced lung function at early ages with a small subset of air pollutants: NO_2 and NO_x (Bougas et al., 2018; Morales et al., 2015); benzene (Morales et al., 2015), PAHs (Jedrychowski et al., 2015), PM_{10} (Cai et al., 2020; Mortimer et al., 2008) and carbon monoxide (CO) (Mortimer et al., 2008).

Most studies have used lung function tests assessed by spirometry as indicators of lung health, but very few studies have explored potential lung function biomarkers. Club Cell secretory protein (CC16) is a protein that is believed to play an important role in the lung epithelial fluid due to its anti-inflammatory and antioxidant properties (Madsen et al., 2008). It is predominantly secreted into the airways by pulmonary club cells and non-ciliated epithelial cells, yet is measurable in circulation. Existing evidence shows that low levels of CC16 are associated with a decline in forced expiratory volume in 1 second (FEV_1) in patients with COPD (Park et al., 2013). Also, in a longitudinal study, low CC16 concentrations in childhood have been found to be a predictor for lung function growth deficits in children (Guerra et al., 2015). It has therefore been suggested that circulating CC16 levels could be potentially used as an early biomarker of lung health (Broeckaert and Bernard, 2000; Lakind et al., 2007). In addition, in a recent study, air pollution exposure at birth has been associated with reduced CC16 levels from childhood into adult life (Beamer et al., 2019). Therefore, as CC16 has previously been shown as a predictor of lung function in children, and also has been shown to be affected by air pollution exposure, we set to explore if CC16 could be a potential mediator in the relationship between air pollution exposure and lung health. To our knowledge, no studies have examined the effects of both pre- and postnatal air pollution exposure on CC16 in children, and CC16 with multiple lung function tests during childhood.

The present study aims to analyse whether exposure to a range of air pollutants, both *in utero* and throughout childhood, affects lung function from 4 to 11 years of age, and if CC16 is a potential mediator within this relationship.

2. Materials and methods

2.1. Study population

We included 487 mother-child pairs from the INMA (INfancia y Medio Ambiente) Sabadell population-based birth cohort. Participants were recruited between 2004 and 2006. Women who visited the hospital in their first trimester of pregnancy, and who met the inclusion criteria, were asked to participate. In total, 657 agreed. The inclusion criteria to be featured in the study were as follows: the pregnant women and their children must reside in the Sabadell area; they must be over 16; must have a singleton pregnancy; must not have used any form of assisted reproduction; must be planning to deliver their child in the hospital used in the study; and must not have any problems with communication.

For the purpose of the present study, we selected those participants with at least prenatal air pollution estimations (N = 619) and at least one lung function test conducted during childhood, leading to a study population of 487 children (Fig. 1). Of these, 360 had lung function tests at age 4 years; 400 at age 7 years; 362 at age 9 years; and 291 at age 11 years. 287 children had information on exposure to air pollution (during pregnancy at least) and information on serum CC16 levels at age of 4 years.

The INMA study has been approved by the Ethical Committees of the Municipal Institute of Medical Research (IMIM) and the ethical committees of the Hospital of Sabadell. Pregnant women received both oral and written information about the study, and written informed consent was acquired from the parents of all children.

2.2. Pre- and postnatal air pollution exposure

Air pollution levels were estimated during the prenatal period and yearly until age 11 of the child by the development of Land Use Regression (LUR) models, as previously described elsewhere (Aguilera et al., 2008; Eeftens et al., 2012). Briefly, levels were measured in 57 sites in the Sabadell area to map out the concentration gradient of various pollutants: nitrogen oxides (NO_2, and NO_x), particulate matter (PM) with diameters of $\leq$2.5 μm ($PM_{2.5}$), diameters of $\leq$10 μm (PM_{10}), and diameters between 2.5 and 10 μm (PM_{coarse}); and absorbance of $PM_{2.5}$ (the reflectance of $PM_{2.5}$ filters). In addition, traffic load was monitored within the Sabadell area. LUR modelling was used to build multiple regression models using traffic, geographic and monitoring data from the several locations. This method enables air pollution levels to be predicted at unmeasured sites where the same traffic and geographical information is available (Hoek et al., 2008). LUR models were created for each pollutant using every measurement site, enabling the average annual air pollution exposure to be computed at each individual's residential address.

All of the participating women in the study had birth addresses within the geographical area in which air pollution was monitored. Levels of individual air pollution exposure were estimated using a combination of real air pollution measurements from the Sabadell study area with variables from the geographic information system (GIS) ArcGIS 9.1 (ESRI, Redlands, California, USA), including population density, traffic, land use, elevation and topography. These measurements were used to develop LUR models using the European Study of Cohorts for Air Pollution Effects (ESCAPE) framework. Long-term air pollution exposures for each address were temporally adjusted using routine monitoring station data which allowed yearly exposures to pollutants to be estimated at each residential address (Eeftens et al., 2012).

For the present study, three periods of exposure to air pollution were defined: i) prenatal period, which includes the average exposure of the 1st, 2nd and 3rd trimesters of pregnancy, ii) preschool period, which includes the average exposure between 1 and 4 years of life of the child, and iii) school period, which includes the average exposure between 5 and 11 years of life of the child.

2.3. CC16 levels

At the age of 4 years, peripheral blood samples were collected using venepuncture, performed by a qualified nurse. CC16 levels in blood serum were measured using a commercially available enzyme-linked immunosorbent assay kit (ELISA) from BioVendor (Modrice, Czech Republic). Only one sample was below the lowest standard and was re-

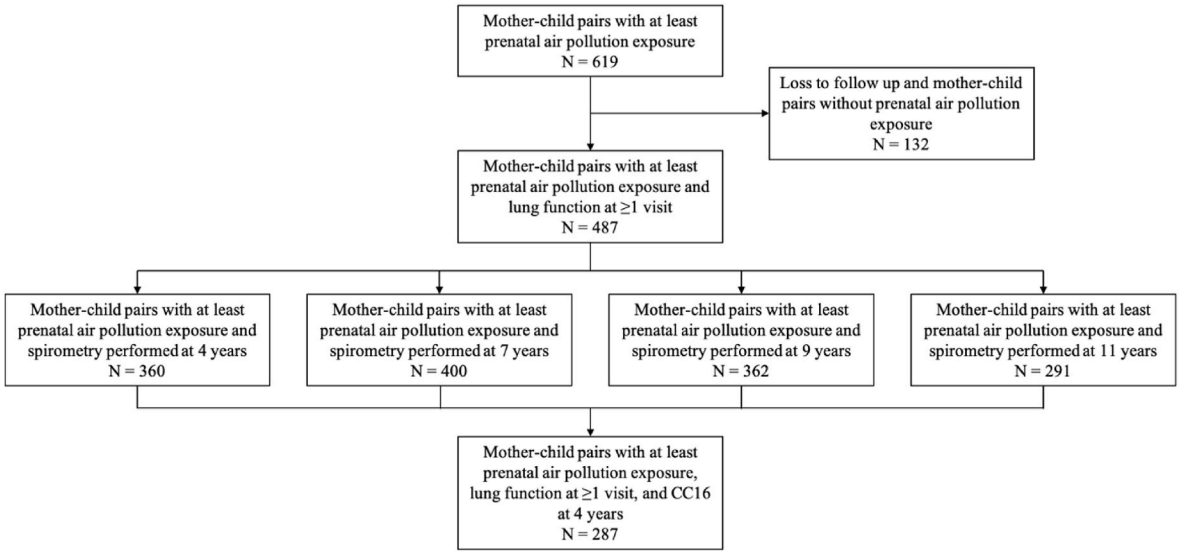

Fig. 1. Flowchart of the included study population.[a]
[a]N, total number; CC16, club cell secretory protein.

assigned half of the lowest standard value times the dilution factor (i.e., 0.785 ng/mL). No sample had CC16 levels above the highest standard. Intra-plate coefficient of variability (CV) was 4.4% and inter-plate CV was 11.1% (9 plates).

2.4. Lung function tests

At ages 4, 7, 9 and 11, children were invited to conduct spirometry tests to assess lung function. Spirometry tests were performed by a trained nurse, using an EasyOne portable spirometer (EasyOne, NDD Medical Technologies, Zürich, Switzerland) following the American Thoracic Society and the European Respiratory Society guidelines (Miller et al., 2005). We used the following lung function parameters in the study: FEV_1 (mL), forced vital capacity (FVC, mL), forced expiratory flow at 25–75% of the FVC (FEF_{25-75}, mL/s) and the FEV_1/FVC ratio (%). All children included in the present study had at least one acceptable manoeuvre.

2.5. Covariates

Confounders, potential effect modifiers and other covariates were collected in questionnaires which were completed at several time points during gestation and early life of the child. More detailed information regarding the study characteristics which were obtained through the questionnaires have been described previously (Guxens et al., 2012).

2.6. Statistical analysis

Correlations between time periods of air pollutant exposures were calculated using Pearson correlation coefficients. Distribution of pollutants and lung function measurements were analysed for normality by visual graphical assessment and Shapiro-Wilk test. Associations with air pollutants were assessed with concentrations as continuous variables, as Generalised additive models (GAMs) did not provide evidence of non-linearity, both graphically and statistically (p-Gain>0.05). Multivariable linear regression models were applied to assess the association between pre- and postnatal exposure periods to air pollution and lung function at individual ages in separate cross-sectional models. Exposure during the school period (between 5 and 11 years) was only assessed in relation to lung function at age 11 years to ensure that exposure precedes outcome. Changes in lung function measurements were reported per interquartile range (IQR) of increasing pollutant concentrations from the average of all time periods of air pollution exposure in order to facilitate the interpretability of the coefficients.

To assess longitudinal associations between air pollutant exposures and lung function growth between ages 4 and 11, linear mixed models (LMM) with random subject intercepts to account for the correlation of repeated measures were used. Individuals with at least two lung function measurements were considered. These models included an exposure age interaction term, which can be directly interpreted as the annual rate of change in lung function associated with air pollution exposure. Lower order terms were also included to assess the interaction.

In order to be a mediator, CC16 needed to be associated with the exposures; associated with the outcomes of interest; and, when included as a confounder in the final model, render a previously significant result between the exposure and outcome no longer significant (Imai et al., 2010). We used multivariable linear regression to analyse the association between prenatal and preschool air pollutant levels and CC16 levels at age 4 years; and CC16 levels and lung function at ages 4, 7, 9 and 11 years. CC16 was log-transformed in order to satisfy the assumption of normality. LMM were also used to longitudinally assess if there was an association between CC16 levels at age 4 years and lung function at subsequent ages.

Confounders were selected *a priori* based on variables that had a significant association with the outcome of interest or had been cited in previous literature research. Fixed covariates included child ethnicity (Caucasian vs. other or mixed), child's sex, maternal education level (primary or without education, secondary, or university), maternal smoking status during pregnancy ["yes" if urinary cotinine levels >50 μg/L at 32 weeks' gestation (Aurrekoetxea et al., 2013)], environmental tobacco smoke (ETS) exposure at 14 months (if positively answered to the question "Does anyone smoke who lives in the household?"), and parity (0, 1, or 2+). Time-varying covariates included child's age and height at time of outcome assessment, and ETS exposure at each lung function test. We performed complete case analysis and excluded individuals who had missing covariate data from cross-sectional and longitudinal analyses.

Possible interactions between air pollution and: i) sex, ii) childhood asthma diagnosis or symptoms [if answered "yes" to "Has the child ever been diagnosed with asthma or has taken any medicines for asthma/breathing difficulties in the last 12 months?"], iii) maternal smoking during pregnancy, iv) maternal history of asthma, and v) duration of weeks breastfeeding were examined. If interactions were statistically significant (Wald test $p < 0.1$), stratification analyses were undertaken.

To assess the robustness of our results, we performed a sensitivity analysis including only those children with at least 2 spirometry acceptable and reproducible curves. Finally, with the aim to assess the effect of multiple air pollution exposures together, we obtained a composite score of all air pollutants by introducing all of them in a Principle Component Analyses (PCA). We selected only the first Principle Component (PC) for each time period, obtained by an Eigenvalue cut off of 1.0. We performed all analyses between the 1st PC for each time period and lung function as were performed between individual air pollutants and lung function. All analyses were performed with Stata/IC 16.1 for Mac.

3. Results

3.1. Study population characteristics

Complete details of the study population are available in Tables 1 and 2. The majority of mothers were nulliparous (57.5%), and a 17.8% of mothers were exposed to tobacco smoke during pregnancy. The majority of the children were Caucasian (96.9%), half (50.9%) were male and only 2.7% were preterm (Table 1). Compared with excluded participants, mothers included in the analyses were older, had higher levels of education and were of a higher social class, and were less likely to smoke during pregnancy (Table 1). The children included in the analysis were more likely to be Caucasian, had breastfed for a greater number of weeks, and were less likely to have been exposed to environmental tobacco smoke (Table 1). At 11 years, mean FVC and FEV_1 were 2633.4 mL and 2211.7 mL, respectively; mean FEV_1:FVC was 84.2% and mean FEF_{25-75} was 2449.5 mL/s (Table 2). CC16 levels at the age of 4 years had a median value of 7.0 ng/mL and an IQR of 4.0 ng/mL (Table 2). NO_x was the air pollutant with the highest mean concentrations across all time periods, as shown in Supplemental Table S1. Correlations between time periods of all pollutants varied between 0.21 and 0.97 (Supplemental Table S2). Correlations between the same pollutants in different time periods were the strongest between pre-school and school (r = between 0.81 and 0.88). The strongest correlations between different pollutants in the same time periods were between NO_x and NO_2 (r = between 0.92 and 0.97) and PM_{10} and PM_{coarse} (r = between 0.94 and 0.95).

3.2. Air pollutants and lung function at each age of assessment

Exposure to nearly all air pollutants during the prenatal period showed trends of reduced FVC, FEV_1, and FEF_{25-75} at all ages (Table 3a). We observed lower FVC at years 7, 9 and 11 with prenatal PM_{10} and PM_{coarse} exposure (e.g. FVC reduced 67.56 mL in relation to a 2.39 μg/m^3 increase in prenatal PM_{coarse} concentration at 11 years, 95% CI: −111.83 to −23.29 mL); and at 11 years with $PM_{2.5}$ (β = −25.46, 95% CI: −50.62 to −0.30 per IQR increase of 1.12 μg/m^3). We observed lower FEV_1 at 9 and 11 years with PM_{coarse}, and at 11 years with PM_{10} (e.g. FEV_1 reduced 68.89 mL, 95% CI: −109.69 to −28.10 per IQR increase in PM_{10} of 2.39 μg/m^3). We observed lower FEF_{25-75} at 11 years with an IQR increase of 2.39 μg/m^3 in PM_{coarse} (β = −109.09, 95% CI: −199.95 to −18.23). There were no associations between air pollutants and FEV_1:FVC. There was a trend of reduced FVC, FEV_1 and FEF_{25-75} at some ages with preschool air pollution exposure (Table 3b). This trend was statistically significant with PM_{10} and PM_{coarse} and FVC at age 7 years (e.g. FVC reduced 27.25 mL with an IQR increase in PM_{coarse} of 2.39 μg/m^3, 95% CI: −52.03 to −2.48 mL). There were no statistically significant associations between air pollution exposure during the school age time window and lung function at age 11 (Table 3c).

3.3. Longitudinal analyses of air pollutants and lung function growth from ages 4 to 11

Prenatal exposure to most pollutants showed trends of reduced FVC growth (Table 4). The association almost reached statistical significance with a reduction in FVC growth with prenatal exposure to PM_{coarse} (reduced by 1.88 mL per year per 2.39 μg/m^3 PM_{coarse} increase, 95% CI: −4.07 to 0.3 mL) and prenatal PM_{10} (reduced by 1.25 mL per year per 4.16 μg/m^3 PM_{10} increase, 95% CI: −2.58 to 0.08 mL). Prenatal PM_{coarse} and PM_{10} exposure also showed trends of reduced FEV_1 growth, although this did not reach statistical significance. Estimates of the association between the other pollutants and FEF_{25-75} growth showed no clear associations. Conversely, prenatal exposure to air pollutants showed trends of an increased growth in FEV_1/FVC ratio, being this significant for NO_x, $PM_{2.5}$ and $PM_{2.5}$ absorption (e.g. FEV_1/FVC ratio

Table 1
Study population and comparison between included and excluded participants.

Characteristic	Included (N = 487)	Excluded (N = 170)		Total cohort (N = 657)
	%/mean (SD)	%/mean (SD)	p-value	%/mean (SD)
Parent Characteristics				
Maternal age at delivery (years)	31.8 (4.2)	30.2 (4.9)	**<0.001**	31.5 (4.4)
Maternal pre-pregnancy BMI (kg/m^2)	23.8 (4.6)	23.5 (4.1)	0.410	23.7 (4.5)
Maternal education level				
Primary or without education	25.0	40.2	**0.000**	28.9
Secondary	42.8	42.6		42.7
University	32.2	17.2		28.3
Maternal social class				
I + II	22.2	10.6	**0.000**	19.2
III	33.3	21.8		30.3
IV + V	44.6	67.7		50.5
Parity				
0	57.5	51.2	0.089	55.9
1	36.5	39.3		37.2
2+	6.0	9.5		6.9
Maternal history of atopy, yes	31.3	34.1	0.500	32.0
Paternal history of atopy, yes	26.5	23.5	0.432	25.7
Smoking during pregnancy, yes[a]	17.8	29.8	**0.007**	20.4
Child characteristics				
Sex				
Male	50.9	48.9	0.676	50.5
Female	49.1	51.1		49.5
Gestational age (weeks)	39.7 (1.4)	39.4 (2.2)	0.129	39.7 (1.6)
Preterm birth (<37 weeks), yes	2.7	4.7	0.255	3.2
Low birth weight (<2500 gr), yes	4.3	6.9	0.282	4.9
Ethnicity				
Caucasian	96.9	88.8	**0.002**	94.8
Other or mixed	3.1	11.2		5.2
Number of weeks predominant breastfeeding				
0	20.0	33.6	**<0.001**	22.6
>0 - 16	29.8	29.4		29.8
>16 - 24	39.3	34.5		38.4
>24	10.9	2.5		9.3
Number of siblings at birth				
0	58.8	53.0	0.143	57.3
1	35.5	38.7		36.3
2+	5.8	8.3		6.4
Day care attendance before 1 year old, yes	17.7	11.8	0.135	16.8
ETS exposure at 14 months, yes	48.0	67.5	**0.001**	50.8

N, total number; SD, standard deviation; BMI, body mass index; ETS, environmental tobacco smoke.

[a] "Yes" to smoking during pregnancy was calculated if urinary cotinine levels were >50μg/at 32 weeks.

Table 2
Study population characteristics at 4, 7, 9 and 11 years of the child (N = 487).

	4 years		7 years		9 years		11 years	
	N (%)/mean ± SD		N (%)/mean ± SD		N (%)/mean ± SD		N (%)/mean ± SD	
Spirometry available, total N (%)	360 (73.9)		400 (82.1)		362 (74.3)		291 (59.8)	
Sex, female	174 (48.3)		189 (47.3)		182 (50.3)		127 (43.6)	
Age (years)	4.5 ± 0.18		6.8 ± 0.38		9.1 ± 0.65		11.1 ± 0.53	
Height (cm)	106.2 ± 4.5		121.8 ± 5.8		135.0 ± 7.1		145.7 ± 7.5	
Weight (kg)	18.2 ± 2.8		25.3 ± 5.3		33.4 ± 7.9		42.0 ± 10.3	
ETS exposure, yes	163 (45.3)		181 (45.7)		154 (42.7)		99 (34.9)	
Asthma diagnosis or symptoms, yes	3 (0.8)		71 (18.0)		50 (13.9)		21 (7.6)	
Lung function measurements	**N**	**Mean (SD)**	**N**	**Mean (SD)**	**N**	**Mean (SD)**	**N**	**Mean (SD)**
FVC (mL)	360	1000.0 (217.9)	400	1623.1 (273.3)	333	2078.8 (351.9)	291	2633.4 (447.7)
FEV_1 (mL)	360	922.7 (189.2)	400	1393.8 (228.0)	362	1785.5 (303.8)	291	2211.7 (375.6)
FEV_1/FVC (as %)	360	92.8 (7.2)	400	86.2 (6.3)	329	86.5 (6.0)	291	84.2 (5.6)
FEF_{25-75} (mL/s)	360	1274.9 (368.6)	399	1652.0 (439.4)	328	2131.5 (541.7)	291	2449.2 (653.5)
Biomarkers	**N**	**Median (IQR)**						
CC16 (ng/ml)	287	7.0 (4.0)						

N, total number; SD, standard deviation; ETS, environmental tobacco smoke; NA, data not available; FVC, forced vital capacity; FEV_1, forced expiratory volume in 1 s; FEF_{25-75}, forced expiratory flow 25–75%; CC16, club cell secretary protein.

increased by 0.006% per year per 23.9 μg/m^3 NO_x increase, 95% CI: 0.000–0.012%). Generally, prenatal NO_x showed trends of growth in all lung function parameters.

Effects of air pollutants on lung function at each age after adjusting for lung function growth are shown in Supplemental Tables S3a and S3b. FVC and FEV_1 are both reduced at 9 and 11 years with prenatal exposure to PM_{10} and PM_{coarse} (e.g. FVC reduced by 16.24 mL at age 11 with a 2.39 μg/m^3 increase in PM_{coarse}, 95% CI: −27.19 to −5.29 mL), and prenatal FEF_{25-75} generally showed negative trends at each age after adjusting for lung function growth. There was mostly a negative trend between exposure to pollutants and FEV_1/FVC ratio at age 4, but a positive trend with exposure to all pollutants and FEV_1/FVC ratio at age 11 (Supplemental Table S3a). This corresponds with a significant increased growth in FEV_1/FVC ratio between the ages of 4 and 11 with some pollutants shown in Table 4. Overall, there were no clear associations between preschool air pollution exposure and lung function growth in any of the parameters assessed (Supplemental Table S3b).

3.4. CC16 as potential mediator

Although CC16 levels at age 4 years showed mildly inverse associations with some air pollutants with both prenatal and preschool exposure (Table 5), no statistically significant association was observed. In cross-sectional analyses, increasing levels of CC16 at age 4 years showed trends of increased FEV_1, FEF_{25-75} and FEV_1:FVC in nearly all ages, with this increase being statistically significant for FEF_{25-75} at 9 years (β = 220.4, 95% CI: 36.1 to 404.7 per 1-unit increase in log-CC16 levels) (Supplemental Table S4). There were less clear associations between levels of CC16 and FVC. In longitudinal analyses, lung function indicators increased as CC16 levels increased (Fig. 2), being this increase statistically significant for FEF_{25-75} (β = 120.4, 95% CI: 6.3 to 234.5). There was almost a statistically significant association between CC16 levels and FEV_1, and also with FEV_1/FVC ratio (β = 44.3, 95% CI: −1.3 to 90.0, and β = 0.014, 95% CI: 0.000 to 0.029, respectively). Due to CC16 not being significantly associated with prenatal or preschool air pollution levels, mediation analyses were not performed.

3.5. Interactions

None of the interactions tested between air pollutants and: i) sex, ii) childhood asthma diagnosis or symptoms, iii) maternal smoking during pregnancy, iv) maternal history of asthma, or v) duration of weeks breastfeeding reached statistical significance, therefore stratification analysis was not attempted (data not shown).

3.6. Sensitivity analyses

After restricting results to include only those children with at least 2 spirometry acceptable and reproducible curves, results between pollutants and lung function showed the same trends and statistical power was mostly retained (Supplemental Tables S5a, S5b, and S5c). There remained positive trends between CC16 levels at 4 years and lung function tests in cross-sectional analyses (Supplemental Table S6). Results also remained robust in the longitudinal models with CC16 with significant associations still confirmed with FEF_{25-75} (β = 144.6, 95% CI: 24.1 to 265.2 per 1 unit increase in log-CC16) (Supplemental Table S7).

Correlations between individual air pollutants and the 1st PC of each time period are shown in Table S8, and associations between the 1st PCs at each time period and lung function are shown in Supplemental Tables S9–S12. Results showed similar trends between the 1st PCs and lung function as with individual pollutants and in the main analyses. Cross sectional analyses at each age showed more significant negative trends with 1st PC of the prenatal period than those of the preschool and school age time periods (Tables S9a, S9b, S9c). Results for the longitudinal analyses showed similar trends to when evaluating individual pollutants (Tables S10, S11a, S11b), and similarly there was no significant association between CC16 at age 4 and the 1st PCs of prenatal and preschool time periods (Table S12).

4. Discussion

To our knowledge, this is the first study that longitudinally examines the effects of both pre- and postnatal air pollution exposure on lung function, whilst exploring the role of CC16 as a potential mediator in this pathway. Also, we are the first to use a wide range of air pollutants to study the impact of air pollution on lung function in children at several ages. In this study, we identified that prenatal exposure to pollutants PM_{10} and PM_{coarse} were both independently associated with significantly lower levels of FVC at ages 7, 9 and 11, and also FEV_1 at 11 years. In our longitudinal model showing lung function growth over time, prenatal exposure to PM_{coarse} and PM_{10} showed trends of reduced FVC growth from ages 4 to 11. We found that an increase in CC16 concentration at age 4 was significantly associated with an increase in FEF_{25-75} between ages 4 and 11, despite not finding a significant association between air pollution exposure and CC16.

4.1. Air pollution and lung function

Our findings are in line with previous literature which shows that increasing exposure to air pollution is generally associated with

Table 3a
Cross-sectional analyses of FVC, FEV_1, FEF_{25-75} and FEV_1/FVC ratio at ages 4, 7, 9 and 11 with an IQR increase in prenatal air pollutant exposure [a,b].

		FVC (mL)					FEV_1 (mL)					FEF_{25-75} (mL/s)					FEV_1/FVC (as %)				
Visit	Pollutant	N	Coef.	95% CI		p-value	N	Coef.	95% CI		p-value	N	Coef.	95% CI		p-value	N	Coef.	95% CI		p-value
4 years																					
	NO_2	332	−0.30	−28.30	27.71	0.983	332	−6.23	−30.18	17.71	0.609	332	−34.14	−85.14	16.86	0.189	332	−0.43	−1.45	0.60	0.412
	NO_x	328	−10.72	−34.66	13.22	0.379	328	−17.11	−37.54	3.33	0.101	328	**−53.28**	**−96.85**	**−9.72**	**0.017**	328	−0.52	−1.39	0.36	0.249
	PM_{10}	332	−11.19	−38.27	15.89	0.417	332	−11.43	−34.58	11.73	0.332	332	−36.51	−85.84	12.83	0.146	332	0.03	−0.97	1.02	0.960
	$PM_{2.5}$	332	2.18	−12.47	16.83	0.770	332	0.04	−12.50	12.57	0.996	332	−6.68	−33.43	20.07	0.623	332	−0.15	−0.69	0.38	0.572
	$PM_{2.5}$ absorbance	332	−4.99	−25.63	15.66	0.635	332	−12.09	−29.70	5.53	0.178	332	**−42.39**	**−79.82**	**−4.97**	**0.027**	332	−0.57	−1.32	0.18	0.135
	PM_{coarse}	332	−8.11	−33.75	17.53	0.534	332	−8.68	−30.60	13.25	0.437	332	−31.32	−78.05	15.40	0.188	332	−0.01	−0.95	0.93	0.987
7 years																					
	NO_2	352	−15.99	−41.36	9.38	0.216	352	−7.26	−30.31	15.79	0.536	351	6.20	−50.56	62.95	0.830	352	0.34	−0.48	1.17	0.417
	NO_x	346	−20.73	−42.70	1.25	0.064	346	−15.50	−35.57	4.57	0.130	345	−19.14	−68.68	30.40	0.448	346	0.07	−0.65	0.79	0.852
	PM_{10}	**352**	**−27.46**	**−51.53**	**−3.39**	**0.026**	352	−15.78	−37.71	6.15	0.158	351	−1.03	−55.16	53.11	0.970	352	0.44	−0.35	1.23	0.271
	$PM_{2.5}$	352	−10.05	−23.08	2.98	0.130	352	−5.45	−17.30	6.39	0.366	351	−4.69	−33.88	24.50	0.752	352	0.16	−0.26	0.59	0.446
	$PM_{2.5}$ absorbance	352	−13.72	−32.53	5.08	0.152	352	−7.63	−24.72	9.46	0.380	351	−5.04	−47.14	37.06	0.814	352	0.24	−0.38	0.85	0.446
	PM_{coarse}	**352**	**−27.51**	**−50.27**	**−4.75**	**0.018**	352	−18.96	−39.68	1.75	0.073	351	−14.64	−65.87	36.59	0.574	352	0.29	−0.46	1.03	0.446
9 years																					
	NO_2	299	−16.90	−51.36	17.56	0.335	326	−11.47	−40.94	18.00	0.444	295	4.44	−66.15	75.02	0.902	296	0.34	−0.48	1.16	0.417
	NO_x	293	−23.88	−53.86	6.10	0.118	320	−11.87	−37.36	13.61	0.360	289	0.09	−60.75	60.93	0.998	290	0.41	−0.30	1.12	0.258
	PM_{10}	**299**	**−38.01**	**−71.33**	**−4.68**	**0.026**	326	−27.55	−56.34	1.24	0.061	295	−6.76	−75.54	62.01	0.847	296	0.59	−0.20	1.39	0.142
	$PM_{2.5}$	298	−13.82	−31.32	3.67	0.121	326	−11.36	−26.36	3.63	0.137	295	−11.58	−47.62	24.46	0.528	296	0.18	−0.23	0.60	0.385
	$PM_{2.5}$ absorbance	298	−15.94	−40.43	8.54	0.201	326	−7.97	−28.83	12.88	0.452	295	−4.78	−54.84	45.28	0.851	296	0.25	−0.33	0.83	0.401
	PM_{coarse}	**298**	**−37.03**	**−67.55**	**−6.51**	**0.018**	326	**−29.76**	**−56.08**	**−3.44**	**0.027**	295	−18.05	−81.00	44.89	0.573	296	0.44	−0.29	1.17	0.232
11 years																					
	NO_2	244	−47.06	−96.66	2.55	0.063	244	−35.89	−78.81	7.03	0.101	244	−52.20	−153.82	49.42	0.313	244	0.07	−0.85	0.99	0.881
	NO_x	240	−28.28	−69.38	12.81	0.176	240	−24.75	−59.98	10.48	0.168	240	−67.12	−149.66	15.41	0.110	240	−0.06	−0.81	0.69	0.876
	PM_{10}	244	**−75.10**	**−122.46**	**−27.74**	**0.002**	244	**−68.89**	**−109.69**	**−28.10**	**0.001**	244	−90.96	−188.79	6.86	0.068	244	−0.25	−1.14	0.64	0.580
	$PM_{2.5}$	244	**−25.46**	**−50.62**	**−0.30**	**0.047**	244	−20.33	−42.08	1.43	0.067	244	−19.70	−71.34	31.94	0.453	244	0.07	−0.40	0.54	0.770
	$PM_{2.5}$ absorbance	244	−16.18	−50.56	18.19	0.355	244	−15.06	−44.74	14.62	0.318	244	−41.07	−111.03	28.89	0.249	244	−0.10	−0.74	0.53	0.753
	PM_{coarse}	244	**−67.56**	**−111.83**	**−23.29**	**0.003**	244	**−70.17**	**−108.06**	**−32.27**	**0.000**	244	**−109.09**	**−199.95**	**−18.23**	**0.019**	244	−0.49	−1.32	0.34	0.246

NOTE: Bold typeface indicates $p < 0.05$.

FVC, forced vital capacity; FEV_1, forced expiratory volume in 1 s; FEF_{25-75}, forced expiratory flow 25–75%; IQR, interquartile range; N, total number.

[a] IQR values: NO_2 13.6 μg/m^3, NO_x 23.9 μg/m^3, PM_{10} 4.16 μg/m^3, $PM_{2.5}$ 1.12 μg/m^3, $PM_{2.5}$ absorbance 0.54 10^{-5} m^{-1}, PM_{coarse} 2.39 μg/m.^{3}.

[b] Models are adjusted for child's sex, ethnicity, age, and height at time of assessment; maternal educational level, parity, and smoking status during pregnancy; and recent environmental tobacco smoke exposure at 14-month follow-up and each assessment.

Table 3b
Cross-sectional analyses of FVC, FEV_1, FEF_{25-75} and FEV_1/FVC ratio at ages 4, 7, 9 and 11 with an IQR increase in preschool air pollutant exposure [a,b].

		FVC (mL)					FEV_1 (mL)					FEF_{25-75} (mL/s)					FEV_1/FVC (as %)				
Visit	Pollutant	N	Coef.	95% CI		p-value	N	Coef.	95% CI		p-value	N	Coef.	95% CI		p-value	N	Coef.	95% CI		p-value
4 years																					
	NO_2	332	−11.47	−38.22	15.28	0.399	332	−10.95	−33.83	11.92	0.347	332	−29.89	−78.69	18.90	0.229	332	−0.05	−1.03	0.93	0.925
	NO_x	332	−8.82	−28.68	11.04	0.383	332	−7.58	−24.57	9.41	0.381	332	−19.61	−55.85	16.64	0.288	332	0.05	−0.68	0.78	0.895
	PM_{10}	332	−12.67	−41.88	16.54	0.394	332	−13.50	−38.47	11.47	0.288	332	−42.63	−95.83	10.56	0.116	332	−0.13	−1.19	0.94	0.817
	$PM_{2.5}$	332	5.22	−11.41	21.84	0.537	332	0.86	−13.37	15.09	0.905	332	−11.76	−42.11	18.58	0.446	332	−0.38	−0.98	0.23	0.223
	$PM_{2.5}$ absorbance	332	−4.44	−26.65	17.77	0.694	332	−7.28	−26.26	11.70	0.451	332	−24.97	−65.44	15.50	0.226	332	−0.34	−1.15	0.47	0.413
	PM_{coarse}	332	−8.72	−35.75	18.31	0.526	332	−10.78	−33.89	12.32	0.359	332	−39.66	−88.85	9.53	0.114	332	−0.24	−1.23	0.75	0.634
7 years																					
	NO_2	351	−20.88	−45.71	3.96	0.099	351	−9.22	−31.80	13.36	0.422	350	11.45	−44.31	67.21	0.687	351	0.44	−0.38	1.25	0.293
	NO_x	351	−17.75	−36.66	1.16	0.066	351	−7.27	−24.48	9.93	0.406	350	13.20	−29.29	55.69	0.542	351	0.43	−0.19	1.05	0.175
	PM_{10}	**351**	**−27.19**	**−53.80**	**−0.58**	**0.045**	351	−13.56	−37.78	10.65	0.271	350	4.64	−55.23	64.52	0.879	351	0.49	−0.39	1.36	0.274
	$PM_{2.5}$	351	−12.18	−27.51	3.15	0.119	351	−8.21	−22.12	5.71	0.247	350	−5.01	−39.42	29.40	0.775	351	0.13	−0.37	0.64	0.600
	$PM_{2.5}$ absorbance	351	−17.36	−37.76	3.05	0.095	351	−8.34	−26.89	10.21	0.377	350	4.47	−41.37	50.30	0.848	351	0.38	−0.29	1.05	0.263
	PM_{coarse}	**351**	**−27.25**	**−52.03**	**−2.48**	**0.031**	351	−15.54	−38.08	7.01	0.176	350	−1.96	−57.80	53.88	0.945	351	0.40	−0.41	1.22	0.329
9 years																					
	NO_2	299	−8.40	−42.00	25.20	0.623	326	−5.33	−33.92	23.25	0.714	295	−27.38	−96.05	41.29	0.433	296	−0.15	−0.94	0.65	0.714
	NO_x	299	−5.43	−29.88	19.02	0.662	326	−2.88	−23.91	18.15	0.788	295	−12.40	−62.31	37.50	0.625	296	−0.07	−0.65	0.51	0.814
	PM_{10}	299	−15.83	−53.14	21.49	0.405	326	−7.55	−38.84	23.75	0.635	295	−36.46	−112.87	39.96	0.348	296	−0.01	−0.89	0.88	0.986
	$PM_{2.5}$	299	−1.30	−21.07	18.46	0.897	326	−5.81	−22.44	10.82	0.493	295	−30.83	−71.14	9.48	0.133	296	−0.19	−0.65	0.28	0.436
	$PM_{2.5}$ absorbance	299	−12.10	−38.29	14.09	0.364	326	−11.15	−33.29	11.00	0.323	295	−35.22	−88.57	18.12	0.195	296	−0.10	−0.72	0.52	0.753
	PM_{coarse}	299	−18.92	−52.92	15.08	0.274	326	−13.08	−41.38	15.23	0.364	295	−40.09	−109.50	29.32	0.257	296	−0.01	−0.82	0.80	0.982
11 years																					
	NO_2	244	−25.23	−72.95	22.48	0.299	244	−19.33	−60.56	21.91	0.357	244	−47.69	−144.95	49.56	0.335	244	−0.02	−0.90	0.86	0.962
	NO_x	244	−19.44	−54.01	15.13	0.269	244	−13.07	−42.96	16.82	0.390	244	−27.54	−98.08	42.99	0.442	244	0.05	−0.59	0.69	0.871
	PM_{10}	244	−21.03	−74.55	32.50	0.440	244	−27.97	−74.12	18.18	0.234	244	−80.41	−189.11	28.29	0.146	244	−0.49	−1.48	0.50	0.328
	$PM_{2.5}$	244	−9.46	−39.04	20.12	0.529	244	−6.69	−32.25	18.86	0.606	244	−17.53	−77.80	42.74	0.567	244	0.07	−0.48	0.61	0.812
	$PM_{2.5}$ absorbance	244	−19.27	−57.35	18.80	0.320	244	−15.35	−48.25	17.54	0.359	244	−47.99	−125.48	29.50	0.224	244	0.03	−0.67	0.74	0.927
	PM_{coarse}	244	−21.27	−72.03	29.49	0.410	244	−33.28	−76.98	10.41	0.135	244	−91.95	−194.83	10.93	0.080	244	−0.63	−1.56	0.30	0.184

NOTE: Bold typeface indicates $p < 0.05$.
FVC, forced vital capacity; FEV_1, forced expiratory volume in 1 s; FEF_{25-75}, forced expiratory flow 25–75%; IQR, interquartile range; N, total number.
[a] IQR values: NO_2 13.6 μg/m^3, NO_x 23.9 μg/m^3, PM_{10} 4.16 μg/m^3, $PM_{2.5}$ 1.12 μg/m^3, $PM_{2.5}$ absorbance 0.54 10^{-5} m^{-1}, PM_{coarse} 2.39 μg/m.^{3}.
[b] Models are adjusted for child's sex, ethnicity, age, and height at time of assessment; maternal educational level, parity, and smoking status during pregnancy; and recent environmental tobacco smoke exposure at 14-month follow-up and each assessment.

Table 3c
Cross-sectional analyses of FVC, FEV_1, FEF_{25-75} and FEV_1/FVC ratio at age 11 with an IQR increase in school age air pollutant exposure [a,b].

		FVC (mL)				FEV_1 (mL)				FEF_{25-75} (mL/s)				FEV_1/FVC (as %)			
Visit	Pollutant	N	Coef.	95% CI	p-value	N	Coef.	95% CI	p-value	N	Coef.	95% CI	p-value	N	Coef.	95% CI	p-value
11 years																	
NO_2		232	1.73	−47.14	50.61	0.944	232	−4.32	−46.48	37.83	0.840	232	−43.05	−142.11	56.01	0.393	232	−0.23	−1.13	0.68	0.623
NO_x		232	0.43	−40.91	41.77	0.984	232	−5.45	−41.10	30.20	0.764	232	−45.69	−129.40	38.01	0.283	232	−0.24	−1.01	0.52	0.535
PM_{10}		232	7.27	−41.84	56.38	0.771	232	−5.25	−47.61	37.10	0.807	232	−34.94	−134.55	64.67	0.490	232	−0.44	−1.35	0.47	0.339
$PM_{2.5}$		232	4.33	−25.89	34.54	0.778	232	6.29	−19.76	32.35	0.635	232	0.90	−60.46	62.26	0.977	232	0.14	−0.42	0.70	0.630
$PM_{2.5}$ absorbance		232	4.07	−38.69	46.82	0.852	232	−1.54	−38.42	35.34	0.934	232	−39.29	−125.94	47.36	0.372	232	−0.13	−0.92	0.66	0.750
PM_{coarse}		232	11.28	−33.19	55.75	0.618	232	−5.02	−43.39	33.35	0.797	232	−33.82	−124.04	56.40	0.461	232	−0.54	−1.36	0.28	0.196

NOTE: Bold typeface indicates $p < 0.05$.
FVC, forced vital capacity; FEV_1, forced expiratory volume in 1 s; FEF_{25-75}, forced expiratory flow 25–75%; IQR, interquartile range; N, total number.
[a] IQR values: NO_2 13.6 μg/m^3, NO_x 23.9 μg/m^3, PM_{10} 4.16 μg/m^3, $PM_{2.5}$ 1.12 μg/m^3, $PM_{2.5}$ absorbance 0.54 10^{-5} m^{-1}, PM_{coarse} 2.39 μg/m.^{3}.
[b] Models are adjusted for child's sex, ethnicity, age, and height at time of assessment; maternal educational level, parity, and smoking status during pregnancy; and recent environmental tobacco smoke exposure at 14-month follow-up and each assessment.

reductions in lung function (Bougas et al., 2018; Jedrychowski et al, 2010, 2015; Korten et al., 2017). The biological mechanisms that relate air pollution exposure *in utero* with reduced lung function in children are relatively unknown. However, inhaled pollutants from the mother have been found to be transported to tissues on the fetal side of the placenta (Bové et al., 2019) into the amniotic fluid (Barošová et al., 2015), and more recently, to the phagocytes in the placenta (Liu et al., 2021), suggesting that these particles could reach the fetus. Air pollution exposure has been shown to impact organogenesis, which includes the development of the lung (Kajekar, 2007). This effect can be magnified by indirect air pollution effects such as reduced birth weight, prematurity or defective immune system development (Korten et al., 2017). In fact, our results show that prenatal air pollution exposure has a greater impact than postnatal exposure. This could be explained as foetal and germ cells have a higher rate of replication when compared to mature cells, and in addition, they have a higher rate of differentiation and are more susceptible to surrounding changing cells (Leibel and Post, 2016).

Several cross-sectional studies found results with similar trends to ours. In particular, many studies found increasing levels of PM_{10} were associated with a significant reduction on lung function: a birth cohort study by Latzin et al. (2009) only studied this association in newborns, but found that higher levels of exposure to PM_{10} during pregnancy were associated with higher minute ventilation and tidal flows in infants aged 5 weeks. A more recent study found that a 10 μg/m^3 increase in both pre- and postnatal exposure to PM_{10} was associated with reduced FVC% predicted and FEV_1% predicted at age 8 years (Cai et al., 2020). Urman et al. (2014) found reductions of 2.5% in FVC and FEV_1 in children aged 10 to 12 with regional PM_{10} and $PM_{2.5}$ exposure from age 5–7 to time of assessment, but similar to our study, no statistically significant associations were observed for NO_2.

We reported similar trends to previous papers which additionally analysed effects of air pollution on lung function growth in longitudinal analyses: a study which examined exposure from birth until 11 years found that a 1 μg/m^3 increase in PM_{10} was associated with reduced FEV_1 growth longitudinally between ages 3 and 11 (β = −1.37%, 95% CI: −2.52 to −0.23) (Mölter et al., 2013). They did not find this association to be statistically significant when conducting cross-sectional analyses, however they additionally observed a statistically significant association between lifetime exposure to NO_2 and FEV_1 growth between the same ages (β = −0.83%, 95% CI: −1.39, −0.28). Another birth cohort study by Milanzi et al. (2018) examined associations with lifetime exposure to all pollutants that were used in our study apart from NO_x, and found significant reductions in FEV_1 growth between ages 8 and 16 with all pollutants, including PM_{10} (β = −0.20, 95% CI: −0.33 to −0.08) and PM_{coarse} (β = −0.17, 95% CI:−0.28 to −0.06). They additionally found reductions in FEV_1 with a 0.9 μg/m^3 increase in PM_{10} (β = −1.29%, 95% CI −2.31 to −0.26%). They also used prenatal and preschool time windows in their analyses, but had a greater study population size (N = 915), which could explain their more robust results when compared to ours. Despite not finding significant associations with $PM_{2.5}$ in our study, other studies found significant results, such as previously referenced studies (Milanzi et al., 2018; Urman et al., 2014), and a study by Jedrychowski et al. (2010), who found that prenatal $PM_{2.5}$ exposure was associated with a reduction in FEV_1 and, at the highest tertile of exposure, $PM_{2.5}$ was associated with a deficit in FVC in children of 5 years of age.

Various studies have found significant associations with exposure to nitrogen oxides, for which we also observed some trends, despite not finding any statistically significant results. Bougas et al. (2018) found a statistically significant reduction in FEF_{25-75} with prenatal exposure to NO_x, however, similar to our results, did not find this association to remain for FVC, FEV_1, nor the FEV_1/FVC ratio in their overall results, and no association was found between postnatal air pollution and reduced lung function. A study by Morales et al. (2015) reported a significant reduction of FEV_1 at pre-school age associated with prenatal exposure to NO_2 (β = −26.1, 95% CI:−51.9 to −0.2). This study also

Table 4
Adjusted associations of lung function growth between ages 4 and 11 with an IQR increase in air pollution exposure in prenatal and preschool time periods [a,b,c].

Time period	Pollutant	N	Difference in FVC (95% C.I.)	N	Difference in FEV_1 (95% C.I.)	N	Difference in FEF_{25-75} (95% C.I.)	N	Difference in FEV_1/FVC (as %) (95% C.I.)
Prenatal									
NO_2		403	−0.27 (−0.70 to 0.16)	413	−0.05 (−0.40 to 0.31)	402	0.33 (−0.46 to 1.12)	403	0.011 (−0.001 to 0.023)
NO_x		397	0.06 (−0.14 to 0.26)	407	0.13 (−0.03 to 0.30)	397	0.22 (−0.14 to 0.58)	397	**0.006 (0.000 to 0.012)**
PM_{10}		403	−1.25 (−2.58 to 0.08)	413	−0.67 (−1.77 to 0.44)	402	0.79 (−1.68 to 3.26)	403	0.022 (−0.016 to 0.059)
$PM_{2.5}$		403	−1.67 (−4.35 to 1.01)	413	−0.41 (−2.63 to 1.80)	402	2.17 (−2.77 to 7.11)	403	**0.077 (0.002 to 0.152)**
$PM_{2.5}$ abs.		403	1.85 (−5.76 to 9.46)	413	4.10 (−2.16 to 10.37)	402	8.76 (−5.24 to 22.76)	403	**0.224 (0.010 to 0.438)**
PM_{coarse}		403	−1.88 (−4.07 to 0.3)	413	−1.40 (−3.21 to 0.41)	402	−0.21 (−4.26 to 3.84)	403	0.018 (−0.044 to 0.080)
Preschool									
NO_2		402	−0.03 (−0.43 to 0.37)	412	0.08 (−0.25 to 0.40)	402	−0.04 (−0.77 to 0.69)	402	0.002 (−0.009 to 0.013)
NO_x		402	0.00 (−0.16 to 0.17)	412	0.04 (−0.09 to 0.18)	402	0.02 (−0.28 to 0.32)	402	0.001 (−0.004 to 0.005)
PM_{10}		402	−0.19 (−1.64 to 1.26)	412	−0.01 (−1.20 to 1.18)	402	−0.88 (−3.55 to 1.78)	402	−0.004 (−0.044 to 0.037)
$PM_{2.5}$		402	−1.10 (−4.13 to 1.92)	412	−0.41 (−2.90 to 2.07)	402	−1.55 (−7.13 to 4.03)	402	0.046 (−0.040 to 0.131)
$PM_{2.5}$ abs.		402	−1.72 (−9.90 to 6.47)	412	0.14 (−6.58 to 6.87)	402	−3.03 (−18.11 to 12.04)	402	0.110 (−0.120 to 0.341)
PM_{coarse}		402	−0.42 (−2.81 to 1.98)	412	−0.35 (−2.31 to 1.61)	402	−2.30 (−6.71 to 2.11)	402	−0.012 (−0.080 to 0.055)

IQR, interquartile range; FVC, forced vital capacity; FEV_1, forced expiratory volume in 1 s; FEF_{25-75}, forced expiratory flow 25–75%.
[a] Preschool time period: 1–4 years.
[b] IQR values: NO_2 13.6 μg/m^3, NO_x 23.9 μg/m^3, PM_{10} 4.16 μg/m^3, $PM_{2.5}$ 1.12 μg/m^3, $PM_{2.5}$ absorbance 0.54 10^{-5} m^{-1}, PM_{coarse} 2.39 μg/$m.^3$.
[c] Models are adjusted for child's sex, ethnicity, age and height at time of assessment, child's baseline lung function and air pollutant exposure; maternal educational level, parity, and smoking status during pregnancy; and recent environmental tobacco smoke exposure at 14-month follow-up and each assessment.

Table 5
Adjusted associations of CC16 at age 4 years with an IQR increase in average air pollutant exposure during prenatal and preschool time periods (N = 198[a]) [b,c,d].

Pollutant	Prenatal			Preschool		
	% reduction of CC16	95% CI		% reduction of CC16	95% CI	
NO_2	−0.68	−8.72	7.37	0.89	−6.21	7.99
NO_x	1.99	−4.72	8.70	0.34	−4.81	5.49
PM_{10}	0.22	−7.45	7.89	1.13	−6.65	8.92
$PM_{2.5}$	−1.81	−5.67	2.05	−1.35	−5.45	2.75
$PM_{2.5}$ abs	1.16	−4.54	6.86	−0.28	−5.98	5.43
PM_{coarse}	−1.37	−8.54	5.80	−0.12	−7.32	7.07

CC16, club cell secretory protein; IQR, interquartile range; N = total number.
[a] N = 196 for prenatal NO_x.
[b] Preschool time period: 1–4 years.
[c] IQR values: NO_2 13.6 μg/m^3, NO_x 23.9 μg/m^3, PM_{10} 4.16 μg/m^3, $PM_{2.5}$ 1.12 μg/m^3, $PM_{2.5}$ absorbance 0.54 10^{-5} m^{-1}, PM_{coarse} 2.39 μg/$m.^3$.
[d] Models are adjusted for child's sex, ethnicity, age and height at time of assessment; maternal educational level, parity, and smoking status during pregnancy; and recent environmental tobacco smoke exposure at 14-month and 4-year follow-up.

used the Spanish INMA cohort, however, it comprised a larger cohort of 620 children across two different geographical areas, which could explain why our results, although reporting a similar association between NO_2 and FEV_1, did not reach statistical significance. Our results contrast somewhat to findings observed in the ESCAPE study, which showed a significant reduction in FEV_1 at age 6–8 years with exposure to postnatal NO_2, NO_x, $PM_{2.5}$ and $PM_{2.5}$ absorbance, however no significant association was observed with PM_{10} or PM_{coarse} in 5921 children from different EU countries (Gehring et al., 2013). Importantly, this study only examined this relationship in children once in their life between 6 and 8 years, and so were unable to assess if exposure to pollutants affected lung growth throughout their childhood.

4.2. Air pollution and CC16

To our knowledge, we are the first study aiming to evaluate CC16 as a mediator of the relationship between air pollution and lung function, and there are relatively few previous studies which analyse the relationship between air pollution and CC16 levels in children. We could not confirm a statistically significant association between air pollution exposure and CC16 levels at age 4 years, however it is likely that our results lacked statistical power, as our sample size was probably too small to detect the effects of air pollution on CC16 levels. The

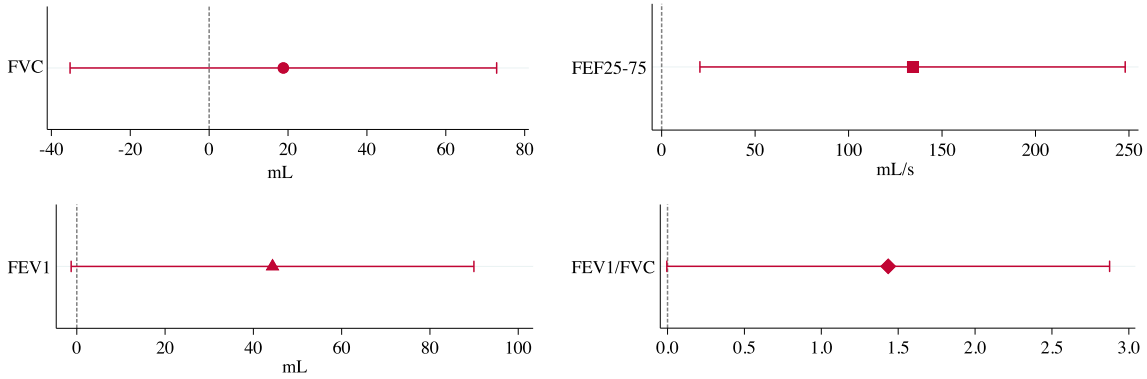

Fig. 2. Longitudinal associations of FVC (mL), FEV_1 (mL), FEF_{25-75} (mL/s) and FEV_1/FVC ratio from ages 4 to 11 with a 1-unit increase in log-CC16 levels at age 4[a]. p=0.495 for FVC; p=0.057 for FEV1; **p=0.021 for FEF_{25-75}**; p=0.051 for FEV_1/FVC ratio.N= 267 for FVC, FEF_{25-75} and FEV1/FVC ratio; N=266 for FEV1.
FVC, forced vital capacity; FEV1, forced expiratory volume in one second; FEF_{25-75}, forced expiratory flow 25-75%; CC16, clara cell secretory protein; N, total number.
[a] Models are adjusted for child's sex, ethnicity, age and height at time of assessment; maternal educational level, parity, and smoking status during pregnancy, and recent environmental tobacco smoke exposure at 14-month follow-up and each assessment.

mechanisms behind how air pollution may impact CC16 levels are also somewhat unclear. CC16 is secreted by Club cells which are present in the epithelial lining of the lungs (Broeckaert and Bernard, 2000). Repeated chronic exposure to harmful environmental factors have been linked to reduced concentrations of CC16 (Rava et al., 2013). Acute exposure to air pollution, conversely, has been linked to increased levels of CC16, perhaps as a biomarker of epithelial damage or as an anti-inflammatory agent in reducing transient local inflammation in the lungs (Lakind et al., 2007). A study by Provost et al. (2014), similar to our study, found no significant association between long term exposure to PM_{10} and serum CC16 measured at age 15 (N = 825), however they found a positive correlation between PM_{10} levels in the week prior to clinical examination and serum CC16 at age 15. Another birth cohort study by Beamer et al. (2019) found that NO_2 levels at birth were associated with a significant decrease in CC16 from ages 6 to 32, yet the authors found no association between NO_2 levels at age 6 and CC16 in the same age range. This study had a larger cohort population (N = 777) in comparison to our study, potentially explaining their more robust results. Future studies examining relationships between air pollutants and CC16 levels in larger cohorts of children are needed to further explore a potential relationship.

4.3. CC16 and lung function

Our results support findings of the few previous studies that have analysed the relationship between CC16 levels and lung function in humans: a study by Zhai et al. (2019) analysed serum CC16 from 707 individuals aged 11 to 32 and similarly found that participants with higher levels of CC16 had significantly higher FEF_{25-75}, FEV_1 and FEV_1/FVC ratio between ages 22 and 32, but no increase in FVC. A study by Guerra et al. (2015) examined the relationship between circulating CC16 between ages 4 and 6 and FEV_1 (N = 1139), and found the lowest tertile of CC16 was associated with a 68 mL deficit in FEV_1 between ages 8 and 16. The association we found in our study between higher levels of CC16 and increased FEF_{25-75}, and almost significant association with FEV_1 and FEV_1/FVC ratio, is incredibly interesting as FEV_1, FEF_{25-75} and FEV_1/FVC ratio are recommended as indicators for obstructive lung disease (Pellegrino et al., 2005). Thus, our results support findings obtained by other studies, which have associated reduced levels of CC16 with increased prevalence of obstructive airway diseases, such as asthma (Shijubo et al., 1999) and COPD (Lomas et al., 2008). Our significant findings with FEF_{25-75} are particularly interesting, as FEF_{25-75} has been suggested as a marker of small airways obstruction (Riley et al., 2015). Small airways are particularly vulnerable to the effects of particle deposition as their narrow lumen predisposes them to obstruction (Burgel et al., 2013). Our significant findings from such a small population size are thus incredibly promising to support existing and future research.

4.4. Strengths and limitations

There were several limitations in our study. First, despite being one of the few birth cohort studies to longitudinally assess the effect of pre- and postnatal air pollution on lung function at several ages, and the first to include the evaluation of CC16 as a potential mediator, our study was probably constrained in its sample size to detect stronger and more consistent effects. Despite the small sample size, the trends observed in our study could suggest that future studies with larger cohort numbers may be able to confirm the hypotheses that we set out to examine. Within our cohort, included children and their mothers differed in some characteristics as compared to those excluded due to either loss of follow up or study design. For instance, the mothers of children included in the analyses were older, had higher levels of education and social class, were less likely to smoke during pregnancy, and children included in the analysis had breastfed for a longer period. However, although these differences may make the results less generalizable to the general population, they should not have any effect on the internal validity of the study. Secondly, due to the nature of the study objectives, performing multiple comparisons potentially increased the chance that the positive associations we found occurred due to chance (Type I error). However, over-reliance on adjustment for multiple comparisons has been argued as counterproductive, and given that most of the associations took the same direction; pollutants are indicators of a similar exposure; and most of the outcomes, as well as exposures, are highly correlated, this could increase the chance of falsely accepting the null hypothesis (Type II error) (Ranganathan et al., 2016). In addition, when pollutants were combined in Principle Component Analyses, most associations remained in the same direction, highlighting the robustness of our results. Although the PCA results were in the same direction as the main analyses, the strength of associations was reduced. This was to be expected as effects of a principle component from 6 individual components will not always fully represent the effects of the components individually (Rauch and Kieser, 2012). Third, despite that spirometry tests were obtained in a healthcare setting by trained medical personnel and adhere to the ERS guidelines (Miller et al., 2005), at the age of 4 years it was more difficult to carry out spirometry testing. This may explain why the associations observed between pollutants and lung function at older ages were not observed at this younger age. In any case, this limitation was overcome in our study by including data at different ages. Our questionnaire data only obtained presence of environmental smoke exposure for each individual at the time of each spirometry visit rather than cumulative years of smoke exposure. However, we obtained accurate smoke exposure data during pregnancy by cotinine levels, and smoke exposure in pregnancy has been suggested to have a greater impact on lung function than postnatal smoke exposure (McEvoy and Spindel, 2017). Nevertheless, including smoke exposure during pregnancy (urinary cotinine >50 μg/mL) in the models did not modify coefficients by over 5%. The sample size in which we tested for a relationship between air pollution and lung function was N = 487, yet there was a smaller N of 200 with data on CC16 in which we tested for a relationship between air pollution and CC16, and CC16 and lung function. However, no population characteristics significantly differed between those with CC16 data and those without (data not shown). In addition, there remained an association between air pollution and lung function when restricting the analysis to those only with data on CC16 (data not shown). We analysed the data using complete case analysis, rather than computing missing data using a method such as multiple imputation. However, the proportion of missing data in covariates included in the model was very low (between 0% and 4.3%), and there is little to gain from imputing covariates with missing data <5% (Lee et al., 2016). Finally, another limitation is that personal air pollution data was not measured, but estimated; personal measurements would improve the accuracy of exposure measurement (e.g. by also taking into account indoor air pollution) and also our ability to detect statistically significant associations. However, by the time INMA Sabadell data was collected, individual pollution monitors were expensive and difficult to use in epidemiological studies of the scale of such study (Hoek, 2017). In addition, LUR modelling catches very small-scale spatial variability in pollutant exposures, and it is a reliable and widely used method in epidemiological studies (Eeftens et al., 2011; Wang et al., 2013). Despite the listed limitations, our study had many strengths. We are one of the few studies to analyse the longitudinal effects of both pre- and postnatal air pollution, as the majority of other studies analysed only postnatal longitudinal effects. Furthermore, we are one of the few studies to measure postnatal exposure from birth, allowing us to estimate total lifetime exposure. In addition, using objective measurements of both exposure and outcome improved the accuracy of our results, in comparison to other studies that analysed the effects of air pollution on respiratory health using patient-reported symptoms, such as breathlessness or wheeze. By using spirometry as an objective measurement of lung function, we minimised the chance of reporting or recall bias. Finally, our study reported no selection bias, as mothers were recruited

at normal antenatal appointments with no prior knowledge of their air pollution exposure at their current address.

5. Conclusion

Our results suggest that pre- and postnatal exposure to air pollution reduces lung function in children, particularly prenatal exposure. Analysis at ages 7, 9 and 11 showed that exposures to PM_{10} and PM_{coarse} during pregnancy had the most consistent effects on reduced lung function, but not exposure at school age. Furthermore, although the small study population prevented us from testing the potential mediating effect of CC16 in these associations, our results provided further evidence of the link between reduced levels of CC16 at pre-school age and reduced lung function in childhood. More research and studies with greater statistical power examining the role of CC16 in the relationship between air pollution and lung function are required.

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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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2021.111900.

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