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Ambient air pollution and inflammation-related proteins during early childhood

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ABSTRACT

Background and aim: Experimental studies show that short-term exposure to air pollution may alter cytokine concentrations. There is, however, a lack of epidemiological studies evaluating the association between long-term air pollution exposure and inflammation-related proteins in young children. Our objective was to examine whether air pollution exposure is associated with inflammation-related proteins during the first 2 years of life.

Methods: In a pooled analysis of two birth cohorts from Stockholm County (n = 158), plasma levels of 92 systemic inflammation-related proteins were measured by Olink Proseek Multiplex Inflammation panel at 6 months, 1 year and 2 years of age. Time-weighted average exposure to particles with an aerodynamic diameter of <10 μm (PM_{10}), <2.5 μm ($PM_{2.5}$), and nitrogen dioxide (NO_2) at residential addresses from birth and onwards was estimated via validated dispersion models. Stratified by sex, longitudinal cross-referenced mixed effect models were applied to estimate the overall effect of preceding air pollution exposure on combined protein levels, "inflammatory proteome", over the first 2 years of life, followed by cross-sectional protein-specific bootstrapped quantile regression analysis.

Results: We identified significant longitudinal associations of inflammatory proteome during the first 2 years of life with preceding $PM_{2.5}$ exposure, while consistent associations with PM_{10} and NO_2 across ages were only observed among girls. Subsequent protein-specific analyses revealed significant associations of PM_{10} exposure with an increase in IFN-gamma and IL-12B in boys, and a decrease in IL-8 in girls at different percentiles of proteins levels, at age 6 months. Several inflammation-related proteins were also significantly associated with preceding PM_{10}, $PM_{2.5}$ and NO_2 exposures, at ages 1 and 2 years, in a sex-specific manner.

Conclusions: Ambient air pollution exposure influences inflammation-related protein levels already during early childhood. Our results also suggest age- and sex-specific differences in the impact of air pollution on children's inflammatory profiles.

1. Introduction

Air pollution is a serious public health issue globally. Particulate air pollution has been associated with increased morbidity including chronic and acute respiratory diseases, cardiovascular diseases, and lung cancer (Dominski et al., 2021; Lee et al., 2021). Previous studies have

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shown that children are particularly sensitive to the adverse effects of air pollution and that exposure during infancy is associated with increased risk of asthma, allergy and impaired lung function up to school age (Gruzieva et al. 2012, 2013; Schultz et al., 2012), adolescence (Gehring et al., 2015; Schultz et al. 2016a, 2016b), and even young adulthood (Wang et al. 2021a, 2021b). Our recent findings suggest that early-life exposure to air pollution might be associated with lung function changes already at the age of 6 months (Lundberg et al., 2022). Nevertheless, the understanding of involved mechanisms remains limited. Findings from experimental models suggest that oxidative stress, inflammation, and mitochondrial dysfunction may contribute to adverse health effects of particulate exposure (Cassee et al., 2013; Niranjan and Thakur, 2017). Epidemiological studies on air pollution exposure and blood markers of systemic inflammation have so far been largely based on adult populations and demonstrated mixed results (Tsai et al., 2019; Xu et al., 2022).

Only very few epidemiological studies investigated the association between air pollution and levels of inflammatory biomarkers in children. Positive associations have been observed between long-term air pollution exposure and increased serum levels of IL-6, IL-10, as well as IL-8 levels in nasal lavage (Barraza-Villarreal et al., 2008; Calderón-Garcidueñas et al., 2013; Klumper et al., 2015). In contrast, several other pediatric studies reported a lack of association with inflammatory markers (Armijos et al., 2015; Brown et al., 2012; Li et al., 2019c). Most of the existing studies on children included school-age children or adolescents, and data on young children are scarce. Prenatal exposure to moderate levels of air pollution may lead to changes in cord blood cytokine levels in healthy infants, including reduced IL-10, as well as increased IL-1β and IL-6 (García-Serna et al., 2022; Latzin et al., 2011). Furthermore, lower production of IL-6, TNF-α, and IL-10 in cord blood mononuclear cells from newborns prenatally exposed to higher levels of $PM_{2.5}$ has been demonstrated in newborns from the US Project Viva cohort (Hahn et al., 2021). The inconsistency in previous findings may partially be attributed to methodological differences (i.e., sample size, model specifications, exposure assessment) and to the actual levels of exposure to air pollutants.

Additionally, the inflammatory response to air pollution may vary depending on the child's sex. For example, in the Swedish birth cohort BAMSE, air pollution exposure during the first year of life was positively associated with IL-6 only in boys, whereas it was negatively associated with IL-2 among girls (Gruzieva et al., 2017). Adult studies have also shown that the expression of inflammatory proteins following air pollution exposure differed by sex (Cabello et al., 2015; Hoffmann et al., 2009).

The present study aims to expanding the limited knowledge on whether air pollution exposure induced changes in systemic inflammatory patterns can be detected already during infancy and early childhood. Also, unlike most previous studies focusing on a single or a limited number of inflammatory markers measured at one point in time, we considered a wide range of inflammatory markers measured repeatedly throughout early childhood, allowing detailed characterization of inflammatory profiles individually as well as combined, i.e. as "inflammatory proteome".

2. Methods

2.1. Study design and population

The present study is based on a pooled analysis of two Swedish birth cohorts: the Etiological Mechanism of air pollution effects on the Infant Lungs (EMIL) and the Born into Life (BiL) cohort. The EMIL cohort was set up to investigate biological mechanisms behind the adverse respiratory health effects of air pollution in children during early life. Parents of children born from 2014 to 2017 in Stockholm city, identified through the Swedish birth register, were invited to participate in the study. The recruitment strategy focused on households residing on streets with low or high air pollution concentration. Low air pollution was defined as particulate matter with a diameter of <10 μm (PM_{10}) levels below 35 μg/m^3 as 90-percentile of daily averages, while high air pollution was defined as PM_{10} levels exceeding 50 μg/m^3 as 90-percentile of daily averages, which is the current air quality standard in the European Union. The exclusion criteria were parents with poor comprehension of the Swedish language; planning to move within 6 months; child with a severe disease that could interfere with the investigations; twins; premature birth (<37 weeks); or low birth weight (<2500 g). At the age of 3 months, parents answered a baseline questionnaire on demographic and residence characteristics, parental smoking habits, and past medical history of the child, both parents, and siblings. Follow-up questionnaires were submitted when the child was 1- and 2-years-old. Further, the children underwent clinical examinations, including blood sampling at ages 6 months (n = 92), 1 (n = 101) and 2 years (n = 100). A total of 108 children provided blood sample at least once. The same protocol was followed, and the same team of research nurses collected all blood samples. Also, if the child had symptoms indicating infection at the blood sampling day, the procedure was postponed until the child recovered.

The Born into Life cohort aims to investigate how conditions before, during and after pregnancy affect health during early childhood (Smew et al., 2018). The Born into Life cohort recruited women from the large cohort study LifeGene, who became pregnant and lived in Stockholm County (Almqvist et al., 2011). The children were born between 2011 and 2013 and informed consent was given by both parents. Similar to the EMIL birth cohort, children in the Born into Life cohort were followed up at 6 months, 1 and 2 years of age with parental questionnaires and clinical examinations. Blood sampling for protein analyses was carried out at 1 year (n = 43), and 2 years follow-up (n = 41). A total of 50 children provided blood sample at least once.

Ethical approvals for the EMIL and Born into Life cohorts and the analyses performed in this study were obtained from the Regional Ethics Review Board, Karolinska Institutet, Stockholm, Sweden. All caregivers provided written informed consent.

2.2. Air pollution exposure assessment

A wind model and a Gaussian dispersion model were used to calculate annual levels of ambient air pollutants at a grid of 35 m for addresses in the more densely populated areas of Stockholm, such as urban areas, and 100 m or a 500 m resolution in less densely populated rural parts. In addition, the Operational Street Pollution Model, OSPM (www.au.dk/OSPM) was used to calculate the dispersion of air pollutants in street canyons. As input to the dispersion modeling, emission inventories for Stockholm and Uppsala counties for the years 2011, 2015 and 2020 were used. To obtain air pollutants levels for years in between linear regression was used for interpolation of the model calculations.

The databases consist of local and regional emission data from e.g. road traffic, shipping, industrial facilities and domestic heating (Segersson et al., 2017). Depending on the source sector, the emissions are described as point, area or line sources. However, for some sectors the emissions are based on regional statistical data and are distributed spatially on a regular grid, using relevant spatial proxies, such as e.g. harbors and residential areas. For both the emission databases and as a framework for running the wind- and dispersion models the Airviro Air Quality Management System (https://www.airviro.com/airviro/modules/) was used. To obtain total concentrations, annual average long-range contributions based on continuous measurements at a regional background station, were added to the locally modelled concentrations. Finally, time-weighted average levels of PM_{10}, $PM_{2.5}$, NO_2 were calculated at the residential addresses from birth up to the date of biosampling at 6 months, 1-year and 2-year follow-ups, respectively, based on residential history and estimated annual levels adjusted for short-term variations using urban background monitor measurements.

2.3. Outcome assessment

Inflammation-related proteins were measured in plasma samples from EMIL and Born into Life cohorts collected at 6 months (only in EMIL), 1 and 2 years of age. Samples were analyzed with the multiplex Proximity Extension Assay (PEA), developed by Olink® (Olink Proteomics, Uppsala, Sweden), using the Inflammation panel (www.olink.com). In summary, the PEA uses a pair of protein-specific antibodies attached to oligonucleotides, whose ends are complementary to each other, to simultaneously quantify 92 proteins in one sample (Assarsson et al., 2014). Once the antibodies bind to the target protein, the oligonucleotides hybridize and are amplified through quantitative Polymerase Chain Reaction (qPCR). The qPCR cycle threshold (Ct) for the amplified product is proportional to the initial amount of protein in the sample (Lundberg et al., 2011). The Ct values are then normalized, and the final results are expressed in Olink Proteomics' unique unit, Normalized Protein eXpression (NPX) in a log2 scale (Assarsson et al., 2014).

We also verified the randomization success by confirming that the frequency of values below the lower limit of detection (LOD) and the first 5 principal components of the biomarkers did not differ between the well plates. Quality control was applied to all 92 inflammatory proteins included in the assay. Samples with no measurements and proteins with >25% of values below LOD were removed. For the remaining proteins, values below LOD were imputed to LOD/square root of 2 (Finkelstein and Verma, 2001). A total of 75 and 73 proteins with >75% of samples above LOD in the EMIL and Born into Life cohorts, respectively, were included in the present analyses, comprising 78 unique proteins.

2.4. Statistical analyses

All statistical analyses were conducted with Stata software (version 16.1; StatCorp, USA). Due to similar study design and sampling strategy of the two included cohorts, we pooled the datasets to increase the sample size and statistical power. We examined the association of plasma inflammation-related protein levels with air pollution exposure averaged from birth up to the date of respective biosampling at 6 months, 1 and 2 years of age (referred to as 0–6 months, 0–1 year and 0–2 years).

Firstly, we combined all proteins into one outcome variable (referred to as "inflammatory proteome"), for each clinical visit (i.e., biosampling at 6 months, 1 year and 2 years), and created a protein name variable (referred to as "protein id") to identify specific proteins. In other words, we transformed 78 protein variables from wide format to one "inflammatory proteome" variable in long format. The inflammatory proteome variable was then fitted into a longitudinal cross-referenced mixed effect model to estimate the association between air pollution exposure and all inflammation-related proteins in the panel measured up to 2 years of age. Using PM_{10} as an example, the PM_{10} exposure and visit were modelled as fixed effects, whereas the identification number of a subject, and the specific protein were modelled as random effects. Further, we included interaction terms between visit and PM_{10}, a random slope between specific protein and PM_{10}, a random slope between specific protein and visit at 1 year (using the visit at 6 months as reference), and a random slope between specific protein and visit at 2 years. Stratified by sex, for subject i, visit j, and protein k, the outcome inflammatory proteome was modelled as:

$$Protein_{ijk} = \beta_o + u_i + u_k + (v_k + \beta_1)PM10_{ij} + (v_{1k} + \beta_2)Visit_{1i} + \beta_3 Visit_{1i}PM10_{ij} + (v_{2k} + \beta_4)Visit_{2i} + \beta_5 Visit_{2i}PM10_{ij} + e_{ijk}$$

Where:

- $Protein_{ijk}$ is the log-base-2 of the NPX in subject i in visit j in protein k.
- β_0 is the overall mean of the protein concentrations of the inflammatory proteome.
- PM_{10ij} is the lifetime PM_{10} exposure before the visit j, in subject i.
- β_1 is the population mean change in the protein concentrations in the inflammatory proteome, for each unit change of PM_{10}.
- v_k is the random slope on PM_{10} at protein k. In other words, the model allows each protein to have its own relationship between protein concentrations and PM_{10}, and the coefficient on PM_{10} would represent the average of these relationships.
- $Visit_{1i}$ is the visit at 1 year of age for subject i.
- β_2 is the population mean difference in the protein concentrations in this inflammatory proteome in the visit at 1 year of age, compared to the visit at 6 months of age.
- v_{1k} is the random slope on the visit at 1 year of age at protein k, compared to the visit at 6 months of age. In other words, the model allows each protein to have its own change between 6 months and 1 year of age, and the coefficient would represent the average of these changes.
- β_3 is the coefficient for the interaction term between the visit at 1 year and PM_{10} compared to the visit at 6 months.
- $Visit_{2i}$ is the visit at 2 years of age for subject i.
- β_4 is the population mean difference in the protein concentrations in this inflammatory proteome in the visit at 2 years of age, compared to the visit at 6 months of age.
- v_{2k} is the random slope on the visit at 2 years of age at protein k, compared to the visit at 6 months of age. In other words, the model allows each protein to have its own change between 6 months and 2 years of age, and the coefficient would represent the average of these changes.
- β_5 is the coefficient for the interaction term between the visit at 2 year and PM_{10}, compared to the visit at 6 months.
- u_i is the random effect of subject i.
- u_k is the random effect of protein k.
- e_{ijk} is the residual of the outcome due to individual variation.
- The number of subjects, i, and the number of proteins, k, are different at different visit j.

The outcome of the regression models is the protein expression. The models allowed for the possibility that different proteins may have different expression levels and included a protein-specific random effect.

To understand to what extent the association of inflammatory proteome with air pollutants may be influenced by a mixed nature of included proteins, we conducted a sensitivity analysis, excluding well-recognized anti-inflammatory proteins (i.e., IL-10 and TGF-beta) from the definition of the inflammatory proteome.

Next, we applied bootstrapped quantile regression models to estimate the association between air pollution exposure and each of the 78 proteins separately, stratifying by sex and age, at specific quantiles of protein levels (i.e., 25th, 50th, and 75th percentiles). Quantile regression is known to be robust to outliers, skewness, and heteroscedasticity on the response variable (Koenker and Hallock, 2001). All results are presented as regression coefficients and 95% confidence intervals (CI) across the interquartile range (IQR) of air pollution levels averaged over the period 0–6 months, to enhance comparability of the effects from the different exposure periods.

Potential confounders were selected based on literature review and data availability, and included sex, birth weight, gestational age, maternal education, parental smoking, older sibling, furry pet, mold or dampness in the residence, season of birth, and breastfeeding. Final models were only adjusted for time indicator (i.e., clinical visit at 6 months, 1 and 2 years), as the rest of the variables did not consistently influence the air pollution and inflammation-related proteins associations, with exception for child's sex, therefore all results were stratified by sex. We further conducted sensitivity analyses including additional adjustments for daily average temperature at the date of biosampling, as well as mode of delivery. In addition, we excluded one subject born

prematurely (i.e., <37 weeks of gestation) from the Born into Life cohort to evaluate potential impact on the estimated associations. Multiple testing was accounted for in the quantile regression analyses by controlling the false discovery rate (FDR) at 5%, implementing the Benjamini-Hochberg adjustment (Strimmer, 2008). FDR-corrected p-value<0.05 was considered statistically significant, unless otherwise specified.

3. Results

3.1. Descriptive statistics of the study population

The study population comprised 158 children. The distribution of selected background characteristics, the inflammatory proteome, and time-weighted average of air pollution exposure (PM_{10}, $PM_{2.5}$ and NO_2) for both cohorts are summarized in Table 1. Children in the EMIL and Born into Life cohorts were similar in terms of proportion of girls (42% vs. 40%), preterm birth (0% vs. 2%), mode of delivery (66% vaginal vs. 68% vaginal), and maternal smoking during pregnancy (0% in both). Yet, the EMIL cohort had more older siblings in family (53% vs. 70%) and more mothers with university degrees or higher (89% vs. 76%). The distribution of air pollution concentrations in the EMIL and Born into Life cohorts was comparable. There were generally strong correlations ($r > 60$) between $PM_{2.5}$, PM_{10} and NO_2 exposures calculated as time-weighted averages from birth to the date of biosampling at respective age (Supplementary Table S1).

Table 1
The distribution of descriptive characteristics of children in the EMIL and Born into Life cohorts.

Characteristics	EMIL cohort N = 108	Born into Life cohort N = 50
Inflammatory proteome, median (q25, q75)		
6 months follow-up	7.0 (4.1, 9.2)	NA
1 year follow-up	6.9 (4.1, 9.1)	6.5 (3.1, 8.4)
2 years follow-up	6.7 (4.0, 9.0)	6.5 (3.3, 8.6)
PM_{10} 0–6 ms,[a] median (q25, q75), μg/m^3	13.9 (12.1, 18.0)	NA
PM_{10} 0–1 yr,[b] median (q25, q75), μg/m^3	13.4 (12.1, 17.4)	13.9 (12.6, 15.2)
PM_{10} 0–2 yrs,[c] median (q25, q75), μg/m^3	12.9 (11.9, 17.1)	13.6 (12.6, 15.1)
$PM_{2.5}$ 0–6 ms,[a] median (q25, q75), μg/m^3	5.6 (5.1, 6.7)	NA
$PM_{2.5}$ 0–1 yr,[b] median (q25, q75), μg/m^3	5.5 (5.1, 6.3)	5.8 (5.3, 6.3)
$PM_{2.5}$ 0–2 yrs,[c] median (q25, q75), μg/m^3	5.5 (5.1, 6.2)	5.7 (5.4, 6.1)
NO_2 0–6 ms,[a] median (q25, q75), μg/m^3	18.0 (12.3, 27.9)	NA
NO_2 0–1 yr,[b] median (q25, q75), μg/m^3	18.0 (12.1, 25.6)	11.9 (8.5, 16.8)
NO_2 0–2 yrs,[c] median (q25, q75), μg/m^3	15.5 (11.4, 24.3)	12.5 (8.2, 15.7)
Female sex	45 (42%)	20 (40%)
No older sibling	57 (53%)	35 (70%)
Preterm birth (i.e., <37 weeks)	0 (0%)	1 (2%)
Mode of delivery:		
Vaginal	71 (66%)	34 (68%)
Cesarean	26 (24%)	9 (18%)
Vacuum extractor	11 (10%)	6 (12%)
Forceps	0 (0%)	1 (2%)
Mothers with university degree or higher	96 (88.9%)	38 (76%)
Maternal smoking during pregnancy	0 (0%)	0 (0%)
Anyone in the household smoked at the time of:		
6 months follow-up	9/92 (9.8%)	NA
1 year follow-up	7/101 (6.9%)	0/43 (0%)
2 years follow-up	6/100 (6.0%)	1/41 (2.4%)

NA=Not available
The unit of inflammatory proteome is Normalized Protein eXpression value, NPX (log2-scale)
[a] Time-weighted average exposure during 0-6 months of age.
[b] Time-weighted average exposure during 0-1 year of age.
[c] Time-weighted average exposure during 0-2 years of age

3.2. Longitudinal associations between air pollution exposure and inflammatory proteome

In our main analysis combining quantitative proteomics data, we identified significant longitudinal associations of the inflammatory proteome profile with air pollution exposure during the first 2 years of life among girls (Table 2). For PM exposures, we observed inverse association with inflammatory proteome, while it appeared to have positive direction with exposure to NO_2. Among boys, we identified significant associations of the inflammatory proteome profile with PM_{10} during the first 6 months of age and $PM_{2.5}$ exposure during the first 2 years of life, but not with NO_2. The identified age-specific associations had consistent directions across ages though significantly different magnitude based on the Wald tests for the null hypothesis that the coefficients of the interaction terms between the age groups and exposure indicator are jointly equal to 0 ($p < 0.05$).

For both boys and girls, the coefficients of the random slope between PM exposures (PM_{10} and $PM_{2.5}$) and specific proteins were generally small. Therefore, the impact of the variation of protein-specific slopes on the PM – inflammatory proteome association is likely minimal.

However, the coefficient of the random slope between NO_2 exposure and specific protein was large, compared to the NO_2 – inflammatory proteome estimate, especially for boys (random slope: 0.08 vs. estimate: 0.10). Since some proteins may increase and other proteins may decrease in association with NO_2, and most proteins had different slopes, the impact on the inflammatory proteome variation may be due to biological differences across different proteins.

In the sensitivity analysis, we rerun the analysis using redefined inflammatory proteome outcome by removing known anti-inflammatory proteins. The results remained largely unchanged (Supplementary Table S2). Further, additional adjustments for daily average ambient temperature, as well as mode of delivery yielded similar association estimates as our main results (Supplementary Tables S3–S4). Also, analysis restricted to term born children generated similar association estimates (Supplementary Table S5).

3.3. Protein-specific associations with air pollution exposure

Next, we analyzed age- and sex-specific associations of air pollution exposure with each protein separately by means of quantile regression. We observed multiple statistically significant associations of air pollution exposure with proteins concentrations at predefined percentiles across different age and sex strata after adjustment for multiple comparison (FDR p-value<0.05) (Supplementary Tables S6–S7; Supplementary Figs. S1–S2). An IQR increase in PM_{10} exposure levels from birth to 6 months of age was associated with 0.51 unit increase in the 25th percentile of Interferon Gamma (IFN-gamma) and 0.35 unit increase in the 25th percentile of Interleukin-12 subunit beta (IL-12B) among 6-month-old boys, as well as 0.59 unit decrease in the 75th percentile of IL-8 among 6-month-old girls (Fig. 1; Supplementary Table S8), whereas there was no evidence that preceding $PM_{2.5}$ or NO_2 exposure was associated with any of the inflammation-related proteins at the age of 6 months (Supplementary Tables S9–S10).

At the age of 1 year, average PM_{10} exposure from birth to 1 year of age was associated with a decrease in the median of osteoprotegerin (OPG), the 75th percentile of adenosine Deaminase (ADA) and fibroblast growth factor 23 (FGF-23) among girls (Supplementary Table S11). Associations with FGF-23 in girls were also detected in relation to $PM_{2.5}$ and NO_2 exposure during the 1st year of life (Supplementary Tables S12–S13). Further, average $PM_{2.5}$ exposure from birth to 1 year was also linked to a decrease in the 25th percentile of Interleukin 17C (IL-17C) and median of urokinase-type plasminogen activator (uPA) among girls. In 2-year-old girls, PM_{10} exposure from birth to 2 years of age was linked

Table 2
Sex-stratified analysis of longitudinal associations of inflammatory proteome during the first 2 years of age with preceding average air pollution exposure.

	Girls					Boys				
	Estimate	SE	P	95% CI		Estimate	SE	P	95% CI	
PM_{10}										
PM_{10} (age 6 months)	−0.32*	0.05	2.66E-12	−0.41	−0.23	0.12*	0.04	0.01	0.03	0.20
PM_{10} (age 1 year)	−0.23*	0.04	4.48E-07	−0.31	−0.14	0.02*	0.04	0.69	−0.06	0.09
PM_{10} (age 2 years)	−0.16*	0.05	4.14E-04	−0.25	−0.07	0.03*	0.04	0.50	−0.06	0.11
Protein random intercept	9.27	1.49		6.77	12.71	9.16	1.47		6.69	12.55
Protein random slope: PM_{10}	2.45E-13	.		.	.	8.55E-15	4.26E-14		4.91E-19	1.49E-10
$PM_{2.5}$										
$PM_{2.5}$ (age 6 months)	−0.21*	0.04	1.94E-06	−0.29	−0.12	−0.10*	0.04	0.02	−0.17	−0.02
$PM_{2.5}$ (age 1 year)	−0.22*	0.05	8.41E-07	−0.31	−0.14	−0.18*	0.04	1.23E-05	−0.26	−0.10
$PM_{2.5}$ (age 2 years)	−0.13*	0.04	0.003	−0.22	−0.05	−0.18*	0.05	1.08E-04	−0.27	−0.09
Protein random intercept	9.13	1.48		6.65	12.54	9.16	1.47		6.69	12.55
Protein random slope: $PM_{2.5}$	0.005	0.003		0.001	0.02	1.61E-15	7.85E-15		1.14E-19	2.28E-11
NO_2										
NO_2 (age 6 months)	0.26*	0.07	9.52E-05	0.13	0.39	0.10	0.05	0.08	−0.01	0.21
NO_2 (age 1 year)	0.22*	0.07	0.001	0.09	0.35	0.09	0.06	0.12	−0.02	0.20
NO_2 (age 2 years)	0.32*	0.07	1.00E-05	0.18	0.46	0.06	0.06	0.34	−0.06	0.18
Protein random intercept	8.91	1.44		6.50	12.22	8.81	1.42		6.43	12.07
Protein random slope: NO_2	0.06	0.01		0.04	0.09	0.08	0.02		0.06	0.12

The unit of protein is Normalized Protein eXpression value, NPX (log2-scale). All results are adjusted for air pollution exposure, visit (age time points), interaction between exposure and visit, subject id (random effect), protein name (random effect, i.e. protein_id), random slope of specific protein and exposure, and random slope of specific protein and visit. The results are presented per IQR increase in air pollution exposure, corresponding to 5.9, 1.6 and 15.6 μg/m^3 for PM_{10}, $PM_{2.5}$ and NO_2, respectively. Age-specific estimates at age 1 and 2 years were extracted from postestimation (reference: age 6 months).
* These coefficients are significantly different across age groups based on the Wald tests for the null hypothesis that the coefficients of the interaction terms between the age groups and exposure indicator are jointly equal to 0 ($p < 0.05$).

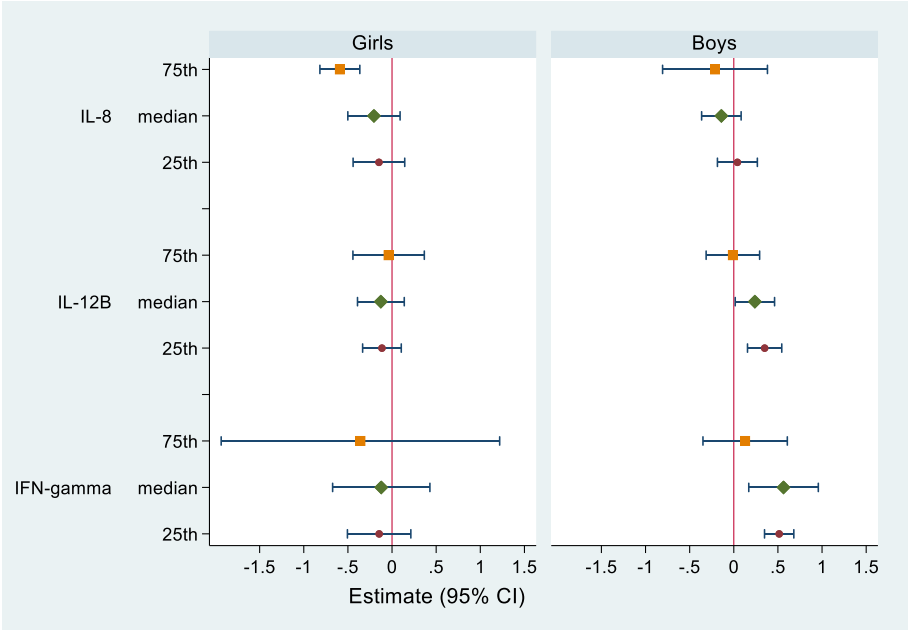

Fig. 1. Sex-specific changes in protein levels at selected percentiles of the protein level distribution in relation to preceding PM_{10} exposure: Summary of FDR-significant associations at 6 months of age.
The exposure is PM_{10} from birth to 6 months of age, the outcomes are selected percentiles of each protein at 6 months of age (based on the EMIL cohort), the unit of protein is Normalized Protein eXpression value, NPX (log2-scale). The results are presented per IQR increase in PM_{10} exposure (5.9 μg/m^3).

to an increase in the 25th percentile of TNF-related apoptosis-inducing ligand (TRAIL) and the median of C–C Motif Chemokine Ligand 11 (CCL11) (Supplementary Table S14). Several of these associations were also nominally significant ($p < 0.05$) across other percentiles of respective proteins. Exposure to $PM_{2.5}$ from birth to 2 years of age was associated with the decrease in the 50th percentile of Interleukin 10 Receptor Subunit Alpha (IL-10RA) and Tumor necrosis factor-beta (TNF-β) among boys (Supplementary Table S15). We observed multiple significant associations of protein levels at 1 and 2 years of age with preceding NO_2 exposure, including the abovementioned proteins also linked to PM exposure, such as CCL11, ADA, TNF-β and OPG (Supplementary Tables S13 and S16). Most of these associations were seen among boys, although several also observed among both sexes.

By looking closer into underlying biological processes, we found that most of the PM_{10}-associated proteins contribute to "Cellular response to cytokine stimulus", "Inflammatory response", "Apoptotic process" and "Cell adhesion", $PM_{2.5}$-accociated – to "Cellular response to cytokine stimulus" and "Inflammatory response", and NO_2-associated – to "Cellular response to cytokine stimulus", "Inflammatory response", "Apoptotic process", "MAPK cascade" and "Chemotaxis" (https://www.olink.com/products-services/target/inflammation). Complete list of relevant biological processes is presented in Supplementary Tables S6 and S7.

4. Discussion

To our knowledge, this is the first study investigating the association between early-life air pollution exposure and a wide panel of repeatedly measured biomarkers of systemic inflammation in children during the first 2 years of life. We found that the overall inflammatory proteome during the first 2 years of life was associated with preceding PM_{10}, $PM_{2.5}$, and NO_2 exposure among girls. In contrast, we identified that the inflammatory proteome profile was associated with $PM_{2.5}$ exposure during the first 2 years of life among boys, but only with PM_{10} during the first 6 months of age and not with NO_2. Subsequent protein-specific analysis revealed several inflammation-related proteins significantly associated with preceding air pollution exposure at different percentiles of proteins levels. Further, the results suggest that child's sex may have a role as an effect modifier in associations between air pollution exposure and inflammation biomarkers.

To the best of our knowledge, no other study has investigated longitudinal changes in the overall inflammatory proteome profile characterized by a combination of a wide range of inflammation-related protein levels measured during early childhood. By applying this novel approach, we found sex-specific and age-varying changes in the childhood inflammatory proteome in relation to air pollution exposure. These results may provide further insights into the biological mechanisms underlying the adverse health effects of air pollution exposure.

Our results support earlier studies about the importance of early life period for the influence of air pollution exposure on the biomarkers of systemic inflammation (Gruzieva et al., 2017; Latzin et al., 2011; Merid et al., 2021). For instance, in the Swedish birth cohort BAMSE, exposure to PM_{10} during the first year of life was linked with increased IL-6 levels among 8-year-old children, as well as increased IL-10 levels among asthmatics (Gruzieva et al., 2017). Calderon-Guarcidueñas et al. compared the serum inflammation biomarkers profile of two groups of children from different areas. The group that lived in Mexico City, where pollution levels were elevated, had higher IL-6 and IL-10 levels, and lower IFN-gamma level than the group that lived in another city with better air quality (Calderón-Garcidueñas et al. 2009, 2013). A German birth cohort study found an association between current exposure to PM_{10} and IL-6 among asthmatic 6-year-old children, however, the authors did not evaluate the effects of early-life exposure (Klumper et al., 2015). A Swiss birth cohort study demonstrated that maternal exposure to PM_{10} during the last trimester of pregnancy was associated with changes in some inflammatory proteins (i.e. reduced IL-10) in cord blood (Latzin et al., 2011). In the present study, it has not been possible to investigate associations with pre- and early postnatal exposure separately due to high correlations in air pollution exposure between the two periods and limited study sizes, as reported in our earlier study, partly based on the EMIL cohort (Lundberg et al., 2022). Our single-protein analysis showed significant associations between PM_{10} exposure and IFN-gamma at the age of 6 months, and significant associations between NO_2 exposure and IL-10 at the age of 1 and 2 years, while we did not observe associations with IL-6, reported earlier. This may partly be attributed to differences between age groups and in the methodology for protein measurement and statistical analysis.

We identified associations of air pollution exposure with several inflammation-related proteins, not included in the previous studies on children. Several studies based on adults reported inverse associations between air pollution exposure and levels of osteoprotegerin (Li et al., 2019b; Saha et al., 2016), which is in line with our results. As OPG is involved in key molecular regulation system for bone remodeling, this finding may contribute to accumulating evidence on the underlying mechanisms behind documented increased risk of low bone mass and osteoporosis in children linked to air pollution exposure (Calderón-Garcidueñas et al., 2013). Air pollution effects on systemic CCL11 and ADA have so far been shown in animal studies (Shih et al., 2018; Thome et al., 2009). CCL11 plays crucial role in eosinophil chemoattraction and activation in asthma pathogenesis, and has been suggested as potentially useful biomarker for the diagnosis and assessment of asthma severity and control (Wu et al., 2014). Emerging evidence suggests the role of air pollution in autoimmune diseases (Zhao et al., 2019). In the present study, we observed association of air pollution exposure with ADA, a degrading enzyme for an immunosuppressive signal, adenosine, playing an important role in immune homeostasis regulation and autoimmune diseases development (Gao et al., 2021). The biomarkers FGF-23, uPA and TRAIL identified in our study represent novel associations in the context of air pollution, thus contributing to the growing body of evidence documenting inflammatory effects of human exposure to air pollution. Interestingly, an earlier study based on the Born into Life cohort reported significant changes in maternal plasma inflammatory proteome profiling during pregnancy, including OPG, TRAIL and IL-10 proteins, that are also identified in the present study (Hedman et al., 2020).

Furthermore, our findings, together with results from previous studies, suggest that the inflammatory response to air pollution may vary depending on the child's sex. We observed that the association between PM_{10} exposure and inflammation-related protein profile tended to be stronger among girls. In the Swedish birth cohort BAMSE, early-life air pollution exposure to PM_{10}, as well as to NO_2 was positively associated with serum IL-6 in boys, while it was negatively associated with IL-2 only in girls (Gruzieva et al., 2017). We had small number of subjects in the sex-specific analysis, and our findings require confirmation in larger studies.

We observed an inverse relationship between PM exposure and overall inflammatory proteome, as well as with several of the identified proteins, which has also been seen previously. In an experimental setting, exposure of bronchial epithelial cells to PM for 72 h resulted in decreased TNF-α, IL-6, and IL-8 compared with cells exposed for shorter periods (i.e., 24 h or 48 h) (Cachon et al., 2014). In another study, volunteers exposed to diesel exhaust and ozone had a lower production of serum TNF-α during the following 22 h (Stiegel et al., 2016). Inverse associations of air pollution exposure with markers of systemic inflammation (e.g., IL-2, IL-8, IL-10, and TNF-α) have also been reported in epidemiological studies (Dobreva et al., 2015; Mostafavi et al., 2015). In a Chinese pediatric population, decreased C3 and C4 levels, as wells as decreased IL6, ICAM1, and TLR2 mRNA levels have been detected in children living in areas of high air pollution compared with children in areas of low air pollution (Li et al. 2019a, 2019c). The long-term duration of the exposure could be partially accountable for the inverse association as demonstrated previously by Dai and coworkers (Dai et al., 2016). Alternatively, the immature immune system of children may make them exhibit different association patterns. Altogether, this may suggest that air pollution exposure has immunomodulatory effects by reducing levels of certain systemic inflammation biomarkers. While some of the proteins were significantly associated with several exposure indicators, other associations were only linked to one of the studied air pollutants. The identified proteins are mainly involved in "Cellular response to cytokine stimulus", "Inflammatory response", "Apoptotic process" biological processes. It remains to be investigated whether non-overlapping associations are pollutant specific.

We found that most detected associations were only significant at one percentile (25th or 75th percentiles), with higher magnitude than the other percentiles. One potential explanation is that for a detected protein, not all children are susceptible to the air pollution exposure, and it is mainly that those children whose protein levels are close to the tail of the distribution were affected. Another potential explanation is that these significant associations with higher magnitude were suffering from the high variability and insufficient data at the tail side, which may result in over or under-estimation of the exposure effect (Wang et al., 2012).

The main strength and novelty of our study lies in its prospective design with repeated objective measurement of inflammation biomarkers over a relatively short time, along with a detailed assessment of individual air pollution exposure. A further strength is the unique age

group of our study population, including children followed during the first two years of life. To the best of our knowledge, no other study has investigated the impact of air pollution on inflammatory biomarkers in this age group. Almost all existing pediatric studies have focused on older children, even though it is recognized that infants may be more susceptible to environmental hazards due to their immature immune system (Simon et al., 2015). Another major strength of the current study is the wide set of inflammation-related protein markers, using well validated methods. Previous studies usually focus on several selected inflammatory proteins, while the current study examined a panel of 92 protein markers, enabling a more thorough and comprehensive exploration of the association between early-life exposure to air pollution and inflammation-related proteins. All measurements for our two cohorts were performed by the same procedures, minimizing misclassification of the outcome. Furthermore, proteins were measured in a multiplex proteomic assay using a PEA technique, which is both specific and sensitive and requires a low sample volume (Assarsson et al., 2014). The interpretation of biological significance of the effect estimates from this study is, however, complicated, since the Olink Proteomics platform measures protein levels in NPX units used for relative quantification only, therefore, the estimates do not translate directly to plasma concentrations.

We conducted detailed assessment of long-term exposure to several air pollution indicators with high geographical resolution for each participating family employing validated dispersion models. Misclassification of exposure might still have occurred, partly because modeling of air pollution may produce unprecise exposure estimates. Additionally, estimated outdoor exposure may not reflect true personal exposure, however, because the exposure was estimated independently from the measurement of biomarkers, potential bias in exposure is likely non-differential, and would generally result in weaker associations. It has been shown that air pollution exposure during pregnancy can influence cytokines in newborns (García-Serna et al., 2022; Latzin et al., 2011). In our study, we were not able to disentangle the role of prenatal versus early postnatal exposure, because most families lived at the same address during pregnancy and after child's birth. Also, most of the parents who changed address between pregnancy and child's birth moved into areas with comparable air pollution levels (Lundberg et al., 2022). Furthermore, we did not take time-activity patterns into account, considering the time that children spent at daycare. However, previous studies from Stockholm and elsewhere have shown that home addresses are well representative of air pollution exposure in young children (Gruzieva et al., 2012; McConnell et al., 2010), largely because the children's kindergartens are often close to their residential areas. It should also be noted that in Sweden children usually start daycare after 1 year of age, implying that air pollution levels at residential addresses should be a good proxy of exposure.

The nonresponse to the invitation to participate in the EMIL and Born into Life cohorts yielded study samples that included fewer smokers and more highly educated parents than in the general population. Therefore, direct generalizability of findings to other populations may be questionable. The response rate during the two-year follow-up was close to 100% in the EMIL cohort and around 80% in the BiL cohort, thus, the internal validity of our study should not be affected. We also acknowledge that the small sample size may have affected the precision of estimates and the study power.

5. Conclusions

Our study contributes to accumulating evidence supporting an impact of ambient air pollution exposure on inflammation reactions that can be detected in early childhood. Moreover, our results suggested age- and sex-specific differences in the impact of early-life air pollution on inflammatory profiles. Given that even small changes in the levels of inflammation-related biomarkers may underlie the increasing risks for respiratory and other health outcomes, these findings have public health implications. Furthermore, the identified inflammation-related biomarkers associated with long-term exposure to air pollution in an area as Stockholm with relatively low air pollution levels may be of relevance for environmental policies.

Credit author statement

Shizhen He: Formal analysis, Software, Visualization, Writing – original draft, Writing – review & editing; **Susanna Klevebro:** Methodology, Writing – review & editing, Supervision; **Gabriel Baldanzi:** Formal analysis, Software; Writing – review & editing; **Göran Pershagen:** Conceptualization, Investigation, Methodology, Writing – review & editing, Funding acquisition; **Björn Lundberg:** Investigation, Writing – review & editing; **Kristina Eneroth:** Methodology, Writing – review & editing; **Anna M Hedman:** Investigation, Writing – review & editing; **Ellika Andolf:** Writing – review & editing, Funding acquisition; **Catarina Almqvist**: Investigation, Writing – review & editing, Funding acquisition; **Matteo Bottai:** Methodology, Software, Supervision, Writing – review & editing; **Erik Melén:** Conceptualization, Methodology, Supervision, Writing – review & editing; **Olena Gruzieva:** Conceptualization, Investigation, Writing – original draft, Writing – review & editing, Supervision, Funding acquisition.

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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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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References

Almqvist, C., Adami, H.O., Franks, P.W., Groop, L., Ingelsson, E., Kere, J., Lissner, L., Litton, J.E., Maeurer, M., Michaëlsson, K., Palmgren, J., Pershagen, G., Ploner, A., Sullivan, P.F., Tybring, G., Pedersen, N.L., 2011. LifeGene–a large prospective population-based study of global relevance. Eur. J. Epidemiol. 26, 67–77.

Armijos, R.X., Weigel, M.M., Myers, O.B., Li, W.W., Racines, M., Berwick, M., 2015. Residential exposure to urban traffic is associated with increased carotid intima-media thickness in children. J Environ Public Health, 713540, 2015.

Assarsson, E., Lundberg, M., Holmquist, G., Björkesten, J., Thorsen, S.B., Ekman, D., Eriksson, A., Rennel Dickens, E., Ohlsson, S., Edfeldt, G., Andersson, A.C., Lindstedt, P., Stenvang, J., Gullberg, M., Fredriksson, S., 2014. Homogenous 96-plex PEA immunoassay exhibiting high sensitivity, specificity, and excellent scalability. PLoS One 9, e95192.

Barraza-Villarreal, A., Sunyer, J., Hernandez-Cadena, L., Escamilla-Nunez, M.C., Sienra-Monge, J.J., Ramirez-Aguilar, M., Cortez-Lugo, M., Holguin, F., Diaz-Sanchez, D., Olin, A.C., Romieu, I., 2008. Air pollution, airway inflammation, and lung function

in a cohort study of Mexico City schoolchildren. Environ. Health Perspect. 116, 832–838.
Brown, M.S., Sarnat, S.E., DeMuth, K.A., Brown, L.A., Whitlock, D.R., Brown, S.W., Tolbert, P.E., Fitzpatrick, A.M., 2012. Residential proximity to a major roadway is associated with features of asthma control in children. PLoS One 7, e37044.
Cabello, N., Mishra, V., Sinha, U., DiAngelo, S.L., Chroneos, Z.C., Ekpa, N.A., Cooper, T. K., Caruso, C.R., Silveyra, P., 2015. Sex differences in the expression of lung inflammatory mediators in response to ozone. Am. J. Physiol. Lung Cell Mol. Physiol. 309, L1150–L1163.
Cachon, B.F., Firmin, S., Verdin, A., Ayi-Fanou, L., Billet, S., Cazier, F., Martin, P.J., Aissi, F., Courcot, D., Sanni, A., Shirali, P., 2014. Proinflammatory effects and oxidative stress within human bronchial epithelial cells exposed to atmospheric particulate matter (PM(2.5) and PM(>2.5)) collected from Cotonou, Benin. Environ. Pollut. 185, 340–351.
Calderón-Garcidueñas, L., Macías-Parra, M., Hoffmann, H.J., Valencia-Salazar, G., Henríquez-Roldán, C., Osnaya, N., Monte, O.C.-d., Barragán-Mejía, G., Villarreal-Calderon, R., Romero, L., Granada-Macías, M., Torres-Jardón, R., Medina-Cortina, H., Maronpot, R.R., 2009. Immunotoxicity and environment: immunodysregulation and systemic inflammation in children. Toxicol. Pathol. 37, 161–169.
Calderón-Garcidueñas, L., Mora-Tiscareño, A., Francolira, M., Torres-Jardón, R., Peña-Cruz, B., Palacios-López, C., Zhu, H., Kong, L., Mendoza-Mendoza, N., Montesinoscorrea, H., Romero, L., Valencia-Salazar, G., Kavanaugh, M., Frenk, S., 2013. Exposure to urban air pollution and bone health in clinically healthy six-year-old children. Arh. Hig. Rada. Toksikol. 64, 23–34.
Cassee, F.R., Heroux, M.E., Gerlofs-Nijland, M.E., Kelly, F.J., 2013. Particulate matter beyond mass: recent health evidence on the role of fractions, chemical constituents and sources of emission. Inhal. Toxicol. 25, 802–812.
Dai, Y., Zhang, X., Zhang, R., Zhao, X., Duan, H., Niu, Y., Huang, C., Meng, T., Ye, M., Bin, P., Shen, M., Jia, X., Wang, H., Yu, S., Zheng, Y., 2016. Long-term exposure to diesel engine exhaust affects cytokine expression among occupational population. Toxicol. Res. 5, 674–681.
Dobreva, Z.G., Kostadinova, G.S., Popov, B.N., Petkov, G.S., Stanilova, S.A., 2015. Proinflammatory and anti-inflammatory cytokines in adolescents from Southeast Bulgarian cities with different levels of air pollution. Toxicol. Ind. Health 31, 1210–1217.
Dominski, F.H., Lorenzetti Branco, J.H., Buonanno, G., Stabile, L., Gameiro da Silva, M., Andrade, A., 2021. Effects of air pollution on health: a mapping review of systematic reviews and meta-analyses. Environ. Res. 201, 111487.
Finkelstein, M.M., Verma, D.K., 2001. Exposure estimation in the presence of nondetectable values: another look. Aihaj 62, 195–198.
Gao, Z.W., Wang, X., Zhang, H.Z., Lin, F., Liu, C., Dong, K., 2021. The roles of adenosine deaminase in autoimmune diseases. Autoimmun. Rev. 20, 102709.
García-Serna, A.M., Martín-Orozco, E., Jiménez-Guerrero, P., Hernández-Caselles, T., Pérez-Fernández, V., Cantero-Cano, E., Muñoz-García, M., Molina-Ruano, M.D., Rojo-Atenza, E., García-Marcos, L., Morales, E., 2022. Cytokine profiles in cord blood in relation to prenatal traffic-related air pollution: the NELA cohort. Pediatr. Allergy Immunol. 33, e13732.
Gehring, U., Wijga, A.H., Hoek, G., Bellander, T., Berdel, D., Bruske, I., Fuertes, E., Gruzieva, O., Heinrich, J., Hoffmann, B., de Jongste, J.C., Klumper, C., Koppelman, G.H., Korek, M., Kramer, U., Maier, D., Melen, E., Pershagen, G., Postma, D.S., Standl, M., von Berg, A., Anto, J.M., Bousquet, J., Keil, T., Smit, H.A., Brunekreef, B., 2015. Exposure to air pollution and development of asthma and rhinoconjunctivitis throughout childhood and adolescence: a population-based birth cohort study. Lancet Respir. Med. 3, 933–942.
Gruzieva, O., Bellander, T., Eneroth, K., Kull, I., Melen, E., Nordling, E., van Hage, M., Wickman, M., Moskalenko, V., Hulchiy, O., Pershagen, G., 2012. Traffic-related air pollution and development of allergic sensitization in children during the first 8 years of life. J. Allergy Clin. Immunol. 129, 240–246.
Gruzieva, O., Bergstrom, A., Hulchiy, O., Kull, I., Lind, T., Melen, E., Moskalenko, V., Pershagen, G., Bellander, T., 2013. Exposure to air pollution from traffic and childhood asthma until 12 Years of age. Epidemiology 24, 54–61.
Gruzieva, O., Merid, S.K., Gref, A., Gajulapuri, A., Lemonnier, N., Ballereau, S., Gigante, B., Kere, J., Auffray, C., Melen, E., Pershagen, G., 2017. Exposure to traffic-related air pollution and serum inflammatory cytokines in children. Environ. Health Perspect. 125.
Hahn, J., Gold, D.R., Coull, B.A., McCormick, M.C., Finn, P.W., Perkins, D.L., Rifas Shiman, S.L., Oken, E., Kubzansky, L.D., 2021. Air pollution, neonatal immune responses, and potential joint effects of maternal depression. Int. J. Environ. Res. Publ. Health 18.
Hedman, A.M., Lundholm, C., Andolf, E., Pershagen, G., Fall, T., Almqvist, C., 2020. Longitudinal plasma inflammatory proteome profiling during pregnancy in the Born into Life study. Sci. Rep. 10, 17819.
Hoffmann, B., Moebus, S., Dragano, N., Stang, A., Möhlenkamp, S., Schmermund, A., Memmesheimer, M., Bröcker-Preuss, M., Mann, K., Erbel, R., Jöckel, K.H., 2009. Chronic residential exposure to particulate matter air pollution and systemic inflammatory markers. Environ. Health Perspect. 117, 1302–1308.
Klumper, C., Kramer, U., Lehmann, I., von Berg, A., Berdel, D., Herberth, G., Beckmann, C., Link, E., Heinrich, J., Hoffmann, B., Schins, R.P.F., Grp, G.I.S., Grp, L. I.S., 2015. Air pollution and cytokine responsiveness in asthmatic and non-asthmatic children. Environ. Res. 138, 381–390.
Koenker, R., Hallock, K.F., 2001. Quantile regression. J. Econ. Perspect. 15, 143–156.
Latzin, P., Frey, U., Armann, J., Kieninger, E., Fuchs, O., Roosli, M., Schaub, B., 2011. Exposure to moderate air pollution during late pregnancy and cord blood cytokine secretion in healthy neonates. PLoS One 6.
Lee, Y.G., Lee, P.H., Choi, S.M., An, M.H., Jang, A.S., 2021. Effects of air pollutants on airway diseases. Int. J. Environ. Res. Publ. Health 18.
Li, J., Wang, T., Wang, Y., Xu, M., Zhang, L., Li, X., Liu, Z., Gao, S., Jia, Q., Fan, Y., Wang, Z., Wu, N., Zhang, X., Dai, Y., Kong, F., Wang, W., Duan, H., 2019a. Particulate matter air pollution and the expression of microRNAs and pro-inflammatory genes: association and mediation among children in Jinan, China. J. Hazard Mater. 389, 121843, 43.
Li, W., Dorans, K.S., Wilker, E.H., Rice, M.B., Ljungman, P.L., Schwartz, J.D., Coull, B.A., Koutrakis, P., Gold, D.R., Keaney Jr., J.F., Vasan, R.S., Benjamin, E.J., Mittleman, M. A., 2019b. Short-term exposure to ambient air pollution and circulating biomarkers of endothelial cell activation: the Framingham Heart Study. Environ. Res. 171, 36–43.
Li, X., Zhang, X., Zhang, Z., Han, L., Gong, D., Li, J., Wang, T., Wang, Y., Gao, S., Duan, H., Kong, F., 2019c. Air pollution exposure and immunological and systemic inflammatory alterations among schoolchildren in China. Sci. Total Environ. 657, 1304–1310.
Lundberg, B., Gruzieva, O., Eneroth, K., Melén, E., Persson, Å., Hallberg, J., Pershagen, G., 2022. Air pollution exposure impairs lung function in infants. Acta Paediatr.
Lundberg, M., Eriksson, A., Tran, B., Assarsson, E., Fredriksson, S., 2011. Homogeneous antibody-based proximity extension assays provide sensitive and specific detection of low-abundant proteins in human blood. Nucleic Acids Res. 39, e102 e02.
McConnell, R., Islam, T., Shankardass, K., Jerrett, M., Lurmann, F., Gilliland, F., Gauderman, J., Avol, E., Künzli, N., Yao, L., Peters, J., Berhane, K., 2010. Childhood incident asthma and traffic-related air pollution at home and school. Environ. Health Perspect. 118, 1021–1026.
Merid, S.K., Bustamante, M., Standl, M., Sunyer, J., Heinrich, J., Lemonnier, N., Aguilar, D., Antó, J.M., Bousquet, J., Santa-Marina, L., Lertxundi, A., Bergström, A., Kull, I., Wheelock Å, M., Koppelman, G.H., Melén, E., Gruzieva, O., 2021. Integration of gene expression and DNA methylation identifies epigenetically controlled modules related to PM(2.5) exposure. Environ. Int. 146, 106248.
Mostafavi, N., Vlaanderen, J., Chadeau-Hyam, M., Beelen, R., Modig, L., Palli, D., Bergdahl, I.A., Vineis, P., Hoek, G., Kyrtopoulos, S., Vermeulen, R., 2015. Inflammatory markers in relation to long-term air pollution. Environ. Int. 81, 1–7.
Niranjan, R., Thakur, A.K., 2017. The toxicological mechanisms of environmental soot (black carbon) and carbon black: focus on oxidative stress and inflammatory pathways. Front. Immunol. 8, 763.
Saha, H., Mukherjee, B., Bindhani, B., Ray, M.R., 2016. Changes in RANKL and osteoprotegerin expression after chronic exposure to indoor air pollution as a result of cooking with biomass fuel. J. Appl. Toxicol. 36, 969–976.
Schultz, E.S., Gruzieva, O., Bellander, T., Bottai, M., Hallberg, J., Kull, I., Svartengren, M., Melen, E., Pershagen, G., 2012. Traffic-related air pollution and lung function in children at 8 years of age: a birth cohort study. Am. J. Respir. Crit. Care Med. 186, 1286–1291.
Schultz, E.S., Hallberg, J., Bellander, T., Bergstrom, A., Bottai, M., Chiesa, F., Gustafsson, P.M., Gruzieva, O., Thunqvist, P., Pershagen, G., Melen, E., 2016a. Early-life exposure to traffic-related air pollution and lung function in adolescence. Am. J. Respir. Crit. Care Med. 193, 171–177.
Schultz, E.S., Hallberg, J., Gustafsson, P.M., Bottai, M., Bellander, T., Bergstrom, A., Kull, I., Gruzieva, O., Thunqvist, P., Pershagen, G., Melen, E., 2016b. Early life exposure to traffic-related air pollution and lung function in adolescence assessed with impulse oscillometry. J. Allergy Clin. Immunol. 138, 930–932 e5.
Segersson, D., Eneroth, K., Gidhagen, L., Johansson, C., Omstedt, G., Nylén, A.E., Forsberg, B., 2017. Health impact of PM10, PM2.5 and black carbon exposure due to different source sectors in Stockholm, gothenburg and umea, Sweden. Int. J. Environ. Res. Publ. Health 14, 742.
Shih, C.H., Chen, J.K., Kuo, L.W., Cho, K.H., Hsiao, T.C., Lin, Z.W., Lin, Y.S., Kang, J.H., Lo, Y.C., Chuang, K.J., Cheng, T.J., Chuang, H.C., 2018. Chronic pulmonary exposure to traffic-related fine particulate matter causes brain impairment in adult rats. Part. Fibre Toxicol. 15, 44.
Simon, A.K., Hollander, G.A., McMichael, A., 2015. Evolution of the immune system in humans from infancy to old age. Proc. Biol. Sci. 282, 20143085.
Smew, A.I., Hedman, A.M., Chiesa, F., Ullemar, V., Andolf, E., Pershagen, G., Almqvist, C., 2018. Limited association between markers of stress during pregnancy and fetal growth in 'Born into Life', a new prospective birth cohort. Acta Paediatr. 107, 1003–1010.
Stiegel, M.A., Pleil, J.D., Sobus, J.R., Madden, M.C., 2016. Inflammatory cytokines and white blood cell counts response to environmental levels of diesel exhaust and ozone inhalation exposures. PLoS One 11, e0152458.
Strimmer, K., 2008. fdrtool: a versatile R package for estimating local and tail area-based false discovery rates. Bioinformatics 24, 1461–1462.
Thome, G.R., Mazzanti, C.M., Ahmed, M., Correa, M., Spanevello, R.M., Maldonado, P.A., Luchese, C., Cargnelutti, D., Morsch, V.M., Duarte, M.M., Fiorenza, A.M., Nogueira, C.W., De Bona, K.S., Moretto, M.B., Da Luz, S.C., Mazzanti, A., Schetinger, M.R., 2009. Activity of ectonucleotidases and adenosine deaminase in rats exposed to cigarette smoke. Inhal. Toxicol. 21, 906–912.
Tsai, D.H., Riediker, M., Berchet, A., Paccaud, F., Waeber, G., Vollenweider, P., Bochud, M., 2019. Effects of short- and long-term exposures to particulate matter on inflammatory marker levels in the general population. Environ. Sci. Pollut. Res. Int. 26, 19697–19704.
Wang, G., Hallberg, J., Um Bergström, P., Janson, C., Pershagen, G., Gruzieva, O., van Hage, M., Georgelis, A., Bergström, A., Kull, I., Lindén, A., Melén, E., 2021a. Assessment of chronic bronchitis and risk factors in young adults: results from BAMSE. Eur. Respir. J. 57, 2002120.
Wang, G., Kull, I., Bergström, A., Hallberg, J., Bergström, P.U., Guerra, S., Pershagen, G., Gruzieva, O., van Hage, M., Georgelis, A., Janson, C., Lindén, A., Melén, E., 2021b.

Early-life risk factors for reversible and irreversible airflow limitation in young adults: findings from the BAMSE birth cohort. Thorax 76, 503–507.
Wang, H.J., Li, D., He, X., 2012. Estimation of high conditional quantiles for heavy-tailed distributions. J. Am. Stat. Assoc. 107, 1453–1464.
Wu, D., Zhou, J., Bi, H., Li, L., Gao, W., Huang, M., Adcock, I.M., Barnes, P.J., Yao, X., 2014. CCL11 as a potential diagnostic marker for asthma? J. Asthma 51, 847–854.
Xu, Z., Wang, W., Liu, Q., Li, Z., Lei, L., Ren, L., Deng, F., Guo, X., Wu, S., 2022. Association between gaseous air pollutants and biomarkers of systemic inflammation: a systematic review and meta-analysis. Environ. Pollut. 292, 118336.
Zhao, C.N., Xu, Z., Wu, G.C., Mao, Y.M., Liu, L.N., Qian, W., Dan, Y.L., Tao, S.S., Zhang, Q., Sam, N.B., Fan, Y.G., Zou, Y.F., Ye, D.Q., Pan, H.F., 2019. Emerging role of air pollution in autoimmune diseases. Autoimmun. Rev. 18, 607–614.