Research

Exposure to Traffic-Related Air Pollution and Serum Inflammatory Cytokines in Children

Olena Gruzieva,[1] Simon Kebede Merid,[1] Anna Gref,[1] Ashwini Gajulapuri,[1] Nathanaël Lemonnier,[2] Stéphane Ballereau,[2] Bruna Gigante,[1] Juha Kere,[3] Charles Auffray,[2] Erik Melén,[1,4,5] and Göran Pershagen[1,5]

[1]Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden
[2]European Institute for Systems Biology and Medicine, CNRS-ENS-UCBL, Université de Lyon, Lyon, France
[3]Department of Biosciences and Nutrition, Karolinska Institutet, Stockholm, Sweden
[4]Department of Paediatrics, Sachs' Children's Hospital, Stockholm, Sweden
[5]Centre for Occupational and Environmental Medicine, Stockholm County Council, Stockholm, Sweden

BACKGROUND: Long-term exposure to ambient air pollution can lead to adverse health effects in children; however, underlying biological mechanisms are not fully understood.

OBJECTIVES: We evaluated the effect of air pollution exposure during different time periods on mRNA expression as well as circulating levels of inflammatory cytokines in children.

METHODS: We measured a panel of 10 inflammatory markers in peripheral blood samples from 670 8-y-old children in the Barn/Child, Allergy, Milieu, Stockholm, Epidemiology (BAMSE) birth cohort. Outdoor concentrations of nitrogen dioxide (NO_2) and particulate matter (PM) with aerodynamic diameter $<10\,\mu m$ (PM_{10}) from road traffic were estimated for residential, daycare, and school addresses using dispersion modeling. Time-weighted average exposures during infancy and at biosampling were linked to serum cytokine levels using linear regression analysis. Furthermore, gene expression data from 16-year-olds in BAMSE ($n = 238$) were used to evaluate links between air pollution exposure and expression of genes coding for the studied inflammatory markers.

RESULTS: A $10\,\mu g/m^3$ increase of NO_2 exposure during infancy was associated with a 13.6% (95% confidence interval (CI): 0.8; 28.1%) increase in interleukin-6 (IL-6) levels, as well as with a 27.8% (95% CI: 4.6, 56.2%) increase in IL-10 levels, the latter limited to children with asthma. However, no clear associations were observed for current exposure. Results were similar using PM_{10}, which showed a high correlation with NO_2. The functional analysis identified several differentially expressed genes in response to air pollution exposure during infancy, including *IL10*, *IL13*, and *TNF*.

CONCLUSION: Our results indicate alterations in systemic inflammatory markers in 8-y-old children in relation to early-life exposure to traffic-related air pollution. https://doi.org/10.1289/EHP460

Introduction

Air pollution exposure has been related to an increased risk of respiratory and allergic disorders in children (Gehring et al. 2015; Nordling et al. 2008; Schultz et al. 2016). Although the exact biological mechanisms are not yet understood, antioxidant and inflammatory responses have been suggested among the key pathways (Esposito et al. 2014; Minelli et al. 2011). The link between the local inflammatory process in the airways and systemic inflammation is considered to involve inflammatory markers secreted by alveolar macrophages and bronchial epithelial cells in response to air pollution exposure, which are capable of entering the bloodstream and stimulating acute-phase reactions (Brook et al. 2010). In addition, direct systemic effects by inhaled particulate matter (PM) that translocates from lungs into the vascular tree, inducing the release of proinflammatory mediators have been demonstrated (Nakane 2012). Many studies have reported associations between short-term exposure to air pollution and acute changes in inflammatory markers, such as increase in pulmonary interleukin (IL-6) measured in exhaled breath condensate (Behndig et al. 2006; Kongerud et al. 2006; Thompson et al. 2010), as well as systemic IL-6 and TNFα levels in adult populations (Tsai et al. 2012), including subgroups with preexisting chronic inflammatory conditions (Rückerl et al. 2007). However, others failed to demonstrate associations with IL-6 (Bräuner et al. 2008; Panasevich et al. 2009; Zuurbier et al. 2011) or TNF-α (Bräuner et al. 2008; Larsson et al. 2013). Studies of long-term exposure to air pollution demonstrate mixed effects on blood markers of systemic inflammation (Chuang et al. 2011; Forbes et al. 2009; Mostafavi et al. 2015; Panasevich et al. 2009). Only very few epidemiological studies investigated the association between traffic-related air pollution (TRAP) and cytokine levels in children. Elevated serum levels of IL-6 in relation to long-term air pollution exposure have been reported (Calderon-Garcidueñas et al. 2013; Klümper et al. 2015), but other studies did not find associations with studied inflammatory markers (Armijos et al. 2015; Brown et al. 2012). The inconsistency in findings may be partially attributed to methodological differences (e.g., sample size, model specifications, exposure assessment), as well as the actual levels of exposure to air pollutants.

A few molecular studies of adults showed that genetic variants in the *IL6* gene may modify effects of both long- and short-term air pollution exposure on inflammatory markers (Ljungman et al. 2009; Panasevich et al. 2013). Further mechanistic support comes from *in vitro* studies showing significantly higher time- and dose-dependent expression of the *IL6* gene (Bach et al. 2015; Liu et al. 2014; Shang et al. 2013; Totlandsdal et al. 2010) in cultured human lung epithelial cells following fine PM exposure. Furthermore, associations were also found with indoor particulate exposure and the level of *IL6* expression in sputum of healthy children (Nazariah et al. 2013). These effects have largely been investigated in studies of short-term exposure to air pollution, there is a lack of mechanistic studies investigating long-term effects. Further investigation of changes in blood inflammation parameters as well as their functional importance is thus needed to elucidate the pathways of disease development caused by

long-term air pollution exposure as well as to identify appropriate biomarkers.

The aim of the present study was to investigate the association of serum inflammatory markers in school-age children with exposure to NO_2, an indicator of combustion-related ambient air pollution, as well as PM_{10}, mostly representing particles mechanically generated from road dust, during different time periods. In addition, we aimed to assess the influence of genetic polymorphisms on the cytokine response to air pollution and to explore potential gene–environment interactions. Finally, we evaluated whether air pollution exposure influenced the expression of genes encoding the studied cytokines, as well as the role of genetic polymorphisms on the mRNA expression response to air pollution exposure.

Methods

Study Population

The Barn/Child, Allergy, Milieu, Stockholm, Epidemiology (BAMSE) study is a population-based birth cohort in which infants were recruited at birth and prospectively followed during childhood and adolescence. A total of 4,089 children born in Stockholm, Sweden, between 1994 and 1996 were included (Wickman et al. 2002). At a median infant age of 2 mo, parents completed a baseline questionnaire that assessed environmental exposures, parental smoking habits, residential characteristics, lifestyle, and parental allergies. When children were approximately 1, 2, 4, 8, 12 and 16 y or age, parents completed questionnaires focusing on symptoms of asthma, rhinitis, and eczema in their children and then-current parental smoking habits (Thacher et al. 2014). Response rates were 96%, 94%, 91%, 84%, 82%, and 78%, respectively. Blood samples were collected at ages 4, 8, and 16 y old from 2,605 (63.7%), 2,470 (60.4%) and 2,547 (62.2%) children, respectively.

For the current study, cytokine levels were measured in a sample of 670 subjects, of whom 473 children were selected based on the availability of other biological data, such as GWAS (genome-wide association study) genotypes. Of these, children with a doctor's diagnosis (DD) of asthma ever up to 8 years old were selected as asthma cases ($n = 237$) and an equivalent number of children with no history of asthma or other allergic diseases (e.g., rhinitis and eczema) served as controls ($n = 236$) (Melén et al. 2013). In addition, about 200 children were selected based on contrasts in air pollution exposure levels during infancy and current exposure at 8 years of age: 105 children with high exposure during infancy ($PM_{10} > 75$th percentile of the exposure range in the cohort) and lower current exposure level at 8 y of age ($PM_{10} < 25$th percentile), and 92 children with lower exposure during infancy and high current exposure at 8 y of age. Exposure during infancy was defined as the average concentration of the respective pollutant at the residential address during the first year of life (from birth to the first-year birthday) using modeled annual levels of pollutants. Current exposure at 8 y of age corresponds to average concentration of air the pollutant during the last 12 mo prior to biosampling at the 8-y-follow-up. Age- and municipality-specific data on the time children were at child care facilities and schools were used to estimate the number of hours spent at home, child care facilities, and schools. Time-weighted average exposure to NO_2 and PM_{10}, respectively, was estimated for each child, depending on the duration of time spent at the different locations. We took changes in residence into account and, in case parents provided two home addresses for the same period (i.e., related to divorce), the child was assumed to have spent 50% of the time at each place. For the first year of life,

only residential addresses were considered because children in Sweden usually do not start daycare before the age of 12 mo.

The Regional Ethical Review Board, Karolinska Institutet, Stockholm, Sweden, approved baseline and all follow-up studies, and the parents of all participating children provided informed consent.

Air Pollution Exposure Assessment

The methodology for calculating individual long-term exposure to locally emitted traffic-related NO_2 and PM_{10} is described in detail elsewhere (Schultz et al. 2016). In brief, the annual mean concentrations of NO_2 and PM_{10} were calculated using a Gaussian air quality dispersion model and a wind model, both part of the Airviro Air Quality Management System (http://airviro.smhi.se). The calculations were performed on a 25-m resolution grid for addresses in the more densely populated areas of Stockholm County, such as urban areas, and 100-m or a 500-m grid in less densely populated areas. Emission databases for NO_2 were available for the years 1990, 1995, 2000, 2002, 2003, 2004, 2006, 2010, and 2015. To obtain NO_2 concentrations for all years during the period of interest, the model calculations were interpolated. PM_{10} model calculations were performed only for the year 2004, when the most complete database was available and applied to all years during the observation period. In addition, a street canyon contribution was added for addresses in the most polluted street segments in the inner city of Stockholm with multistorey houses on both sides, using the Airviro street canyon model (http://airviro.smhi.se).

Outcome Assessment

The levels of inflammatory cytokines IFN-γ, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, TNF-α were measured using electro-chemiluminescence 10-spot MULTISPOT® 96 well plates manufactured by Meso Scale Discovery Multi Array (MSD), using MSD 2,400 instrument (Velásquez et al 2014). The concentrations of serum levels were obtained from the 8-point standard curve, and the units are expressed as picograms per milliliter (pg/ml). For intra-assay variability n = 50, samples were run in duplicates. All analyses were performed according to the manufacturer's assay protocol.

DNA was extracted from peripheral blood leucocytes. The samples were genotyped using the Illumina Human610-Quad BeadChip (Illumina Inc, San Diego, CA, USA), and the genotyping has been described elsewhere (Moffatt et al. 2010).

Data on mRNA gene expression were available in 238 BAMSE children aged 16 y old, of whom 172 had measured serum inflammatory markers at 8 y of age (Gruzieva et al. 2017). Whole blood was collected in PAXGene tubes, and RNA was extracted using the PAXgene Blood RNA kit (QIAGEN). Assessments of extracted RNA yield and quality were performed with Dropsense96 (Trinean) and Tapestation (Agilent) instruments, respectively, discarding two samples. RNA of highest quality was selected for amplification, labeling, and hybridization on Affymetrix HTA 2.0 Genechips using the Affymetrix WT PLUS kit (Affymetrix Inc.) at the European Institute for Systems Biology and Medicine in Lyon. Measures of probes' intensity were then processed at the probe-set level for RMA normalization using Expression Console v1.4. Transcripts were annotated using version 35 of Affymetrix annotation. In addition, automated cell counts were obtained by flow cytometry performed at the Karolinska University Hospital Laboratory in Stockholm, Sweden.

Statistical Analyses

Analysis of cytokine serum levels. The Mann-Whitney U-test was used to compare the distribution of cytokines between the group with asthma and control groups. Linear regression models were applied to estimate effects of air pollution exposure on the levels of serum cytokines, the latter being log-transformed to better approximate normal distribution. Adjustment for body mass index of the child at 8 years of age, exposure to secondhand tobacco smoke (SHS) *in utero*, and/or infancy (defined as maternal smoking at least one cigarette per day at any point of time during the pregnancy and/or at the age of 2 mo), current SHS at 8 and 16 y of age (any of the parents smoked daily at the time of respective follow-up), parental education at baseline (low/medium/high), as well as older siblings did not influence the results and were therefore not included. The final models were adjusted for child's sex, doctor's diagnosis of asthma ever up to 8 y of age, and municipality at birth. Stratified analyses by asthma status, child's sex, and SHS exposure *in utero* and/or child's infancy, as well as analyses including interaction terms representing the product of air-pollutant exposure and stratifying variables, were performed to evaluate potential effect modification. In addition, allelic association of the concentration of IL-6 was tested at 17 SNPs within the *IL6* gene typed in 470 children as part of a GWAS study (Melén et al. 2013). The dbSNP "by gene" function was used to identify all *IL6*-associated SNPs (http://www.ncbi.nlm.nih.gov/variation/view/).

Analysis of cytokine transcript levels. Differential expression of the studied cytokine transcripts in relation with early-life and current air pollution exposure was tested using data obtained as part of the 16-y-old age group follow-up by means of linear regression analysis adjusted for child's sex, DD asthma ever up to 16 y of age, municipality at birth, and measured cell counts.

Furthermore, for IL-6 we evaluated whether the relationship of *IL6* expression with NO_2 exposure differed depending on the genotype status by means of stratified analysis. Analyses of gene expression data were run in "limma" Bioconductor package (Ritchie et al. 2015) in R (version 3.3.1; R Development Core Team).

Air pollution levels were entered as continuous variables without transformation. Estimated air pollution effects were calculated as a change in the log level of an outcome blood parameter for increment of $10 \, \mu g/m^3$ of NO_2 exposure, and $5 \, \mu g/m^3$ of PM_{10} exposure. Change in the outcome was back-transformed and presented as percentage. Statistical analyses were performed with STATA software (release 13.1; StataCorp).

Results

A total of 670 children with serum inflammatory markers measurements at 8 y of age were included in the study. The distribution of selected characteristics and air pollution exposure levels in the study population is provided in Table 1. The mean local contribution above regional background to the children's outdoor TRAP exposure levels during the first year of life, and one year prior to follow-up of the 8-y-old group, respectively, was 16.3 and $8.1 \, \mu g/m^3$ for NO_2, and 6.0 and $4.3 \, \mu g/m^3$ for PM_{10}. The correlations between estimated NO_2 and PM_{10} concentrations in the various time periods were high ($r = 0.97$ and $r = 0.96$ between NO_2 and PM_{10} exposure levels during the first year of life and one year before the follow-up at 8 y of age, respectively). Given the selection frame, we had more children with asthma in the current study sample in comparison with the entire cohort.

Table 2 provides a summary of serum cytokine concentrations in children with and without doctor's diagnosis of asthma ever up to 8 y old. Levels of IL-10 and IL-8 differed between children with and without an asthma diagnosis (Mann-Whitney U-test,

Table 1. Distribution of selected characteristics for the BAMSE children included in the analysis ($n = 670$).

Characteristic	N (%)
Males	367 (54.8)
Doctor's diagnosis of asthma ever up to 8 y	242 (36.1)
Municipality at birth:	
Stockholm	225 (33.7)
Järfälla	174 (26.1)
Solna	190 (28.4)
Sundbyberg	79 (11.8)
Age at follow-up, years, mean ± SD	8.3 ± 0.5
NO_2 during infancy ($\mu g/m^3$), mean/median (min-max)	16.3/15.8 (0.7–40.1)
PM_{10} during infancy ($\mu g/m^3$), mean/median (min-max)	6.0/5.8 (0.3–16.5)
NO_2 at 8 years ($\mu g/m^3$), mean/median (min-max)	8.1/6.2 (0.4–29.9)
PM_{10} at 8 years ($\mu g/m^3$), mean/median (min-max)	4.3/3.5 (0.2–14.3)
Maternal smoking during pregnancy and/or infancy	77 (11.5)
Asthma treatment[a] during the last 12 mo prior to 8-y follow-up	155 (23.2)

[a]Asthma treatment = any treatment for breathing difficulties in the last 12 months.

$p < 0.05$), which may be attributed to the difference in the distribution of cytokine concentrations between these groups, whereas for IL-6 concentration the difference was less clear ($p = 0.09$) (see Figure S1). Pairwise correlation of cytokine levels was moderate to null (Pearson correlation coefficient ranging from $r = 0.48$ for IFNγ and IL-10 to $r = -0.001$ for IL-1β and IL-12p70, data not shown).

A $10 \, \mu g/m^3$ increase of exposure to NO_2 during infancy was significantly associated with an increase of 13.6% (95% CI: 0.8, 28.1%) in IL-6 concentration (Table 3). Positive but not statistically significant associations were also observed with IFNγ and IL-10, while the rest of the tested cytokines tended to be negatively associated with early life NO_2 exposure. Association of current NO_2 exposure at 8 y with IL-6 levels was also positive, but no longer significant. No clear association of current exposure with other considered cytokines was detected. Similar trends were observed for PM_{10} exposure (Table S1).

In stratified analysis by asthma status, early-life NO_2 exposure was significantly associated with increase in IL-6 and IL-10 levels among children with an asthma diagnosis as follows: 28.8% (95% CI: 1.2, 63.8%) and 27.8% (4.6; 56.2%), respectively, although no significant interaction between air pollution exposure during infancy and asthma status was observed (Table 4). However, an inverse relationship between concurrent NO_2 exposure at 8 y of age and concentrations of IL-4 and TNFα was observed in children with asthma (p-value for interaction with DD asthma = 0.03 and 0.003, respectively). Similar results were seen for PM_{10} (Table S2).

Further stratified analysis suggested stronger association of NO_2 exposure during infancy with IL-6 concentrations in boys: 20.8% increase (95% CI: 1.2, 44.2%), in comparison with girls: 6.6% (−9.4; 25.3%), as well as negative association with IL-2 in girls: 24.5% decrease (−41.6; −2.4%), but not in boys: 20.0% increase (−10.3; 60.6%) per $10 \, \mu g/m^3$ increase of NO_2 exposure (Table S3). However, p-values for interaction between NO_2 and sex were not significant ($p > 0.05$). NO_2 exposure at 8 y of age was significantly associated with increased IL-10 levels in children exposed to maternal smoking *in utero* and/or during infancy: 38.4% (95% CI: 9.7, 74.5%) per $10 \, \mu g/m^3$ increase of exposure to NO_2 (p-value for interaction = 0.04). Similar patterns were observed with PM_{10} exposure (Table S4).

In sensitivity analyses, excluding children currently taking asthma medication had little effect on the estimated effects on

Table 2. Distribution of serum inflammatory cytokines concentration stratified by doctor's diagnosis of asthma ever up to 8 y.

Cytokines (pg/Ml)	DD asthma ever up to 8 y ($N = 242$)					No DD asthma ever up to 8 y ($N = 416$)					
	Mean	Median	Min	Max	IQR	Mean	Median	Min	Max	IQR	p-value[a]
IFNγ	7.47	3.97	1.31	100.3	3.12	6.28	4.02	1.65	124.0	2.32	0.54
IL-1β	0.05	0	0	1.85	0	0.09	0	0	8.11	0	0.67
IL-10	0.29	0.23	0.08	2.12	0.13	0.30	0.21	0.05	7.98	0.12	0.03
IL-12p70	0.13	0.11	0	1.48	0.10	0.14	0.11	0	1.59	0.10	0.55
IL-13	0.48	0.47	0	1.98	0.39	0.53	0.50	0	3.17	0.41	0.41
IL-2	0.24	0.15	0	8.71	0.13	0.22	0.15	0	14.09	0.13	0.58
IL-4	0.04	0.03	0	0.87	0.04	0.04	0.03	0	0.49	0.04	0.59
IL-6	0.34	0.27	0.04	2.06	0.21	0.29	0.24	0.06	1.49	0.17	0.09
IL-8	2.03	1.62	0.46	28.81	0.90	2.21	1.77	0.60	27.59	1.13	0.003
TNFα	1.18	1.15	0.50	4.72	0.45	1.17	1.11	0.43	3.56	0.48	0.66

Note: IFN, interferon; IL, interleukin; TNF, tumor necrosis factor.
[a]p-values for differences between children with and without asthma using the Mann-Whitney U-Test.

IL-6 levels (Table S5). The association between early-life traffic-related NO_2 exposure and IL-6 levels was comparable after NO_2 exposure at 8 y of age has been added to the same model, although the estimate was now of borderline statistical significance (Table S6). The correlation between early and current NO_2 exposure levels was moderate ($r = 0.41$). Similar results were observed for PM_{10} exposure (data not shown).

No allelic association was observed between SNPs within the *IL6* gene and levels of IL-6 in blood, nor was evidence of interaction between these SNPs and air pollution exposure on cytokine levels observed (data not shown). Using a sample of 16-y-olds with available transcriptomic data, we examined whether air pollution exposure influenced the expression levels of genes encoding the studied cytokines. Early-life NO_2 exposure was significantly associated with increased expression of *IL10*, *IL13*, and *TNF* but not *IL6* at 16 y of age (Table 5), whereas the association with PM_{10} exposure during infancy appeared to be significant only for *IL10* (Table S7). Current NO_2 and PM_{10} exposures at 16 y of age were linked to differential expression of *IL1β*, *IL12A*, as well as *IL13*. Additional analysis of gene–environment interaction highlighted significant interacting SNP $\times NO_2$ effects on *IL6* expression for one *IL6* SNP, rs2069837 (interaction p-value $= 0.03$) (Table S8). A stratified analysis showed that the effect of NO_2 exposure at 16 y of age on the *IL6* expression was significant only in subjects with the *GG* genotype of rs2069837 ($p = 0.007$) but not in subjects with the AG ($p = 0.13$) genotype.

Discussion

Exposure to TRAP during the first year of life was in this study related to an increased serum level of marker of inflammation IL-6 in children 8 y of age. This association tended to be

Table 3. Associations between NO_2 exposure and blood cytokine levels in the BAMSE children.

Cytokine	NO_2 during infancy		NO_2 at 8 y	
	N	Estimate[a] (95% CI)	N	Estimate[a] (95% CI)
IFNγ	655	11.1 ($-3.3; 27.7$)	644	1.3 ($-8.4; 11.9$)
IL-1β	145	-5.3 ($-55.8; 102.8$)	142	1.3 ($-44.3; 84.2$)
IL-10	655	5.6 ($-6.0; 18.6$)	644	4.1 ($-4.2; 13.1$)
IL-12p70	621	-8.4 ($-24.3; 10.9$)	610	1.8 ($-11.3; 16.9$)
IL-13	585	-1.5 ($-15.5; 14.8$)	576	1.7 ($-9.1; 13.7$)
IL-2	627	-5.5 ($-22.3; 14.9$)	616	0.8 ($-12.4; 15.9$)
IL-4	577	-5.8 ($-24.8; 18.1$)	566	-13.2 ($-25.8; 1.6$)
IL-6	655	13.6 (0.8; 28.1)	644	4.7 ($-3.9; 14.2$)
IL-8	655	0.1 ($-9.7; 10.9$)	644	-5.8 ($-12.5; 1.4$)
TNFα	655	-0.8 ($-7.0; 5.9$)	644	-0.1 ($-4.6; 4.7$)

Note: NO_2, nitrogen dioxide; IL, interleukin; IFN, interferon.
[a]Results are obtained by means of linear regression analyses and presented as percentage change in serum concentration of cytokines per $10 \mu g/m^3$ increase of NO_2 exposure. Adjusted for child's sex, DD asthma ever up to 8 y of age, and municipality at birth.

stronger in boys, as well as in individuals with doctor's diagnosis of asthma. We also found association of air pollution exposure with elevated levels of IL-10 in children with diagnosis of asthma, as well as in children exposed to maternal smoking during pregnancy and/or infancy. Finally, air pollution exposure during infancy also correlated with *IL10*, *IL13*, and *TNF* transcript levels measured at 16 y of age.

Local inflammation together with oxidative stress has been suggested as a potential biological pathway explaining higher susceptibility of individuals with asthma to air pollution exposure (Auerbach and Hernandez 2012). Growing evidence also indicates that systemic inflammation plays an important role in the pathogenesis of asthma (Wouters et al. 2009). We investigated a wide panel of inflammatory biomarkers to evaluate the effects of long-term TRAP exposure on systemic inflammatory status in children. IL-6 has been associated with diseases characterized by systemic reactions (Kishimoto 2006) and mediates inflammation, particularly in the pathogenesis of pulmonary diseases (Pedroza et al. 2011; Rincon and Irvin 2012). Our results are consistent with other studies on adults showing elevated levels of IL-6 in relation to short- (Tsai et al. 2012) and long-term exposure to NO_2 (Chuang et al. 2011; Panasevich et al. 2009), as well as $PM_{2.5}$ (Hajat et al. 2015; Hoffmann et al. 2009). However, only a few epidemiological studies have evaluated influence of exposure to air pollution on markers of systemic inflammation in children. In the German GINI/LISA cohorts, current exposure to NO_2 was associated with higher pro-inflammatory cytokine responsiveness (adjusted means ratio 2.22, 95% CI: 1.22–4.04, for increase in IL-6 per $2.7 \mu g/m^3 NO_2$) in whole blood of 6-y-old children with asthma, with significant interaction between asthma status and NO_2 exposure (Klümper et al. 2015). Similarly, in a pilot study from Mexico, 6-y-old children with a lifetime residency in the highly polluted Mexico City had significantly higher concentrations of IL-6 in comparison with a control group from the municipality of Polotitlán in Mexico State, Mexico, with levels below the current U.S. National Ambient Air Quality Standards (Calderon-Garcidueñas et al. 2013). Both studies had relatively small sample sizes and did not examine the effects of early-life exposure. In contrast, other studies conducted in children reported a lack of association with inflammatory markers (Armijos et al. 2015; Brown et al. 2012). These negative results might partly be explained by the use of proximity to the nearest road as a proxy for individual air pollution exposure, which may result in more exposure misclassification than in studies with more detailed exposure assessment.

Experimental studies showed significantly higher time- and dose-dependent expression of *IL6* in cultured human lung epithelial cells following fine particulate exposure (Bach et al. 2015; Liu et al. 2014; Shang et al. 2013; Totlandsdal et al. 2010). Our finding of elevated serum IL-6 levels could not be supported with

Table 4. Associations between NO_2 exposure and blood cytokine levels stratified by doctor's diagnosis of asthma ever up to 8 y of age in the BAMSE children.

Exposure	Cytokine	Asthmatics		Nonasthmatics		p-value[b] for interaction
		N	Estimate[a] (95% CI)	N	Estimate[a] (95% CI)	
NO_2 during infancy	IFNγ	242	27.5 (−3.7; 68.9)	413	5.3 (−10.0; 23.0)	0.33
	IL-1β	51	−1.3 (−68.1; 206.0)	94	−11.5 (−68.2; 145.9)	0.57
	IL-10	242	27.8 (4.6; 56.2)	413	−2.2 (−15.2; 12.8)	0.61
	IL-12p70	233	−2.1 (−30.9; 38.8)	388	−10.0 (−28.6; −28.6)	0.40
	IL-13	215	0.3 (−20.6; 26.8)	370	−2.7 (−20.3; 18.8)	0.75
	IL-2	234	−22.7 (−47.9; 26.8)	393	2.6 (−17.7; 27.9)	0.57
	IL-4	217	−29.7 (−54.6; 9.0)	360	6.7 (−17.8; 38.7)	0.12
	IL-6	242	28.8 (1.2; 63.8)	413	8.6 (−5.2; 24.3)	0.73
	IL-8	242	−4.1 (−21.2; 16.8)	413	1.8 (−9.9; 14.3)	0.47
	TNFα	242	0.7 (−10.8; 13.7)	413	−0.9 (−8.3; 7.0)	0.25
NO_2 at 8 y	IFNγ	234	−1.7 (−21.1; 22.6)	410	2.1 (−8.9; 14.4)	0.16
	IL-1β	48	114.5 (−18.1; 461.7)	94	−34.8 (−70.6; 44.8)	0.13
	IL-10	234	1.6 (−13.0; 18.7)	410	6.0 (−4.4; 17.6)	0.13
	IL-12p70	225	−8.3 (−29.7; 19.6)	385	8.4 (−8.6; 28.5)	0.17
	IL-13	208	6.2 (−11.5; 27.5)	368	0.5 (−13.4; 16.5)	0.68
	IL-2	226	−11.6 (−34.8; 19.8)	390	5.2 (−10.4; 23.5)	0.29
	IL-4	209	−34.4 (−52.5; −9.3)	357	−1.4 (−18.2; 18.8)	0.03
	IL-6	234	−1.7 (−18.6; 18.7)	410	8.6 (−1.6; 19.8)	0.19
	IL-8	234	−12.6 (−24.7; 1.3)	410	−4.0 (−12.1; 5.0)	0.26
	TNFα	234	−7.7 (−15.9; 1.3)	410	3.9 (−1.8; 9.8)	0.003

Note: NO_2, nitrogen dioxide; IFN, interferon; IL, interleukin; TNF, tumor necrosis factor.
[a]Results are obtained by means of linear regression analyses and presented as percentage change in serum concentration of cytokines per $10 \mu g/m^3$ increase of NO_2 exposure in subjects with asthma and children without asthma, respectively). Adjusted for child's sex, and municipality at birth.
[b]p-value for interaction term representing the product of NO_2 exposure and DD of asthma.

available functional data on gene expression. The fact that serum cytokines were measured in a sample of 8-y-olds and transcriptomic analysis was performed in 16-y-olds might have contributed to the different results. We could, however, see a significant interacting effect of *IL6* SNP, rs2069837, with current NO_2 exposure at 16 y of age on *IL6* expression. However, given the relatively small sample size of individuals with both genetic and mRNA data, our results may be underpowered and should, therefore, be interpreted with caution. Interestingly, this SNP has been suggested as a marker for a susceptibility to inflammation-mediated cancers due to a mechanism altering the *IL6* mRNA expression (Shi et al. 2013; Zhou et al. 2016). Thus, Zhou and colleagues found that the increase of *IL6* mRNA expression was associated with the increasing number of the *G* allele, which is similar to our findings. It is, however, important to note that these analyses were mainly based on Chinese populations, and it remains to be investigated whether the findings can be extrapolated to other ethnic groups.

IL-10, a pleiotropic cytokine released from various types of cells, including mononuclear cells, macrophages, T-lymphocytes, B-lymphocytes, and mast cells, is one of the most important anti-inflammatory cytokines regulating the progression of the immune response by suppressing the production of a number of proinflammatory cytokines, including TNF-α, IL-1β, IL-6, and IL-8 (Chung 2001; Dobreva et al. 2009). Studies have shown that IL-10 is constitutively produced in the lungs and peripheral blood of healthy subjects, which may help prevent the development of inflammation in the healthy state, whereas subjects with asthma are characterized by decreased concentrations of IL-10 (Chung 2001). This finding is in line with our results demonstrating higher levels of serum IL-10 in children without asthma in comparison with children who have had an asthma diagnosis. The increased level of IL-10 after exposure to air pollutants such as diesel exhaust in the airways of patients with asthma indicates that air pollution may induce subtle changes in airway immunobiology (Leem et al. 2005). Furthermore, several studies reported

Table 5. Associations between NO_2 exposure and gene expression in 16-y-old BAMSE children ($N = 238$).

Probe ID	Gene	NO_2 during infancy			NO_2 at 16 y		
		LogFC[a]	p-value	FDR[b] p-value	LogFC[a]	p-value	FDR[b] p-value
TC12001696.hg.1	*IFNG*	0.04	0.41	0.49	0.06	0.29	0.69
TC02002219.hg.1	*IL1B*	−0.06	0.30	0.42	−0.17	0.01	0.09
TC01003766.hg.1	*IL10*	0.09	0.003	0.06	0.05	0.13	0.59
TC03000870.hg.1	*IL12A*	0.04	0.13	0.19	0.08	0.02	0.09
TC05001997.hg.1	*IL12B*	0.03	0.33	0.43	0.02	0.66	0.79
TC05000640.hg.1	*IL13*	0.07	0.01	0.07	0.08	0.02	0.09
TC04001520.hg.1	*IL2*	0.05	0.13	0.19	0.004	0.93	0.93
TC05000641.hg.1	*IL4*	0.03	0.44	0.49	−0.01	0.86	0.93
TC07000137.hg.1	*IL6*	0.001	0.97	0.97	0.02	0.54	0.69
TC04000408.hg.1	*IL8*	−0.01	0.84	0.89	0.01	0.89	0.93
TC6_apd_hap1000036.hg.1	*TNF*	0.09	0.04	0.07	0.06	0.28	0.69
TC06000371.hg.1[c]	*TNF*	0.08	0.04	0.07	0.03	0.51	0.69

Note: IFN, interferon; IL, interleukin; TNF, tumor necrosis factor.
[a]Results are obtained by means of linear regression analyses and presented per $10 \mu g/m^3$ increase of NO_2 exposure. Adjusted for child's sex, DD asthma ever up to 16 y of age, municipality at birth, and measured cell counts. LogFC = logarithm fold-change (one unit of the logFCs translates to a two-fold change in expression).
[b]FDR = false discovery rate correction based on 18 probesets.
[c]Seven alternatives haplotypes assemblies of this TNF probeset are available (apd, cox, dbb, mann, mcf, qbl and ssto) and bear similar log2 intensity values.

increased *IL10* expression and IL-10 plasma levels in children living in highly polluted areas (Calderon-Garcidueñas et al. 2009; Dobreva et al. 2015), which is also supported by *in vitro* studies (Saito et al. 2002).

IL-10 may also play a role in tobacco smoke–induced inflammation. A study of adults showed that IL-10 levels were significantly correlated with urinary 1-OHPG and cotinine, constituting biomarkers of polycyclic aromatic hydrocarbon and SHS exposure, respectively, suggesting that these exposures alter immunological responses leading to increased IL-10 (Leem et al. 2005), which supports our observation of a stronger association between TRAP exposure and IL-10 concentrations in children exposed to tobacco smoke *in utero* and/or early in life. Interestingly, studies considering both tobacco smoke exposure and air pollution exposure demonstrate that tobacco smoke exposure in early life might lead to increased vulnerability of the lungs to the effects of air pollution (Sonnenschein-van der Voort et al. 2012). A synergistic effect of these exposures has also been shown for the molecular changes in the genes involved in the immune function (e.g., significant hypermethylation and decreased expression of *IFNγ* in effector T-cells and *FOXP3* in regulatory T-cells), in comparison with each exposure alone, suggesting a potential mechanism explaining how these exposures may influence the development and progression of asthma and allergies (Kohli et al. 2012).

The strengths of our study include a relatively large and well-characterized study population, detailed long-term air pollution exposure assessment, precise measurements of serum cytokine concentrations, and evaluation of effect modification. Furthermore, the availability of genetic and transcriptomic data provided a unique opportunity to investigate the functional importance of our findings. In addition, because we aimed to investigate the potential effect modification of long-term TRAP exposure by asthma status, using a study sample enriched with children who had been diagnosed with asthma was also advantageous.

The individual exposure estimates for each child were derived from a time- and space-resolved dispersion model with street canyon addition for addresses in the most polluted street segments, which has been successfully applied in a number of previous studies (Gruzieva et al. 2012; Schultz et al. 2016). Validation of dispersion models against actual measurements suggests good model performance in predicting ambient concentrations of NO_2 ($r^2 = 0.71$), PM_{10} and $PM_{2.5}$ ($r^2 = 0.61$) (Eneroth et al. 2006), as well as NO_x ($r^2 = 0.68$) (Korek et al. 2016). Furthermore, to increase the precision in the exposure assessment, we accounted for time-activity patterns, considering the time children spent at home, daycare, and school. We were able to investigate several duration aspects of long-term exposure, such as exposure during the first year of life and current exposure during 12 months prior to blood sampling. The strongest effect was observed in relation to exposure during infancy, which is in line with previous studies indicating that the early-life period represents a critical time window for the development and maturation of several biological systems, including the lung and immune system, and that air pollutants may impair adaptive immune responses, thus contributing to negative health effects throughout childhood (Clark et al. 2010; Gehring et al. 2015; Gruzieva et al. 2012; Schultz et al. 2016). Additionally, the exposure to higher concentrations of combustion-related air pollutants during early childhood than exposure later in life in our study might also have contributed to the higher risks associated with exposure during this period. An alternative explanation might be that the exposure classification method based on home address is more accurate early in life, before children start to go to child care and school. In addition, some misclassification of exposure may have occurred because estimated outdoor exposure levels may not be equivalent to true personal exposure. However, because air pollution exposure was assessed independently from health outcomes and potential confounders, possible misclassification of exposure is likely to be nondifferential, which would tend to attenuate the associations.

Our study also has limitations. One limitation is the lack of longitudinal data on markers of inflammation and gene expression, and such data were available only for air pollution exposure. Another limitation is limited power in some analyses, mainly related to subgroups and interactions. In addition, given the multiple comparisons made, statistically significant results might be due to chance alone. On the other hand, because the significance tests were used to evaluate some *a priori* hypotheses based on existing literature, such as whether air pollution exposure influences IL-6 levels in serum, individual test results were not adjusted for multiple comparisons (Perneger 1998). Another limitation is the difficulty to attribute the observed associations to a specific air pollution component such as (NO_2 or PM_{10}) because of the strong correlation between the levels of these traffic-related pollutants. Finally, we investigated a limited number of inflammatory markers in relation to air pollution exposure without considering patterns or complex interactions between the markers. Future large-scale studies should attempt to explore a broader range of inflammatory cytokines in repeated samples to expand our understanding of underlying mechanisms and interactions between cytokines in the inflammatory response following air pollution exposure.

This study adds to the growing body of evidence demonstrating pro-inflammatory effects of exposure to TRAP. This finding is important from a public-health perspective, given that even slight increases in the levels of inflammatory cytokines may represent a health threat for the population by increasing risks for respiratory and other morbid conditions. Furthermore, the observed association of elevated inflammatory cytokines with long-term exposure to TRAP in an area with comparatively clean air such as Stockholm supports the importance of further lowering urban air pollution levels.

Acknowledgments

References

Armijos RX, Weigel MM, Myers OB, Li W-W, Racines M, Berwick M, et al. 2015. Residential exposure to urban traffic is associated with increased carotid intima-media thickness in children. J Environ Public Health 2015:713540, PMID: 25685160, https://doi.org/10.1155/2015/713540.

Auerbach A, Hernandez ML. 2012. The effect of environmental oxidative stress on airway inflammation. Curr Opin Allergy Clin Immunol 12(2):133–139, PMID: 22306553, https://doi.org/10.1097/ACI.0b013e32835113d6.

Bach N, Bolling AK, Brinchmann BC, Totlandsdal AI, Skuland T, Holme JA, et al. 2015. Cytokine responses induced by diesel exhaust particles are suppressed by par-2 silencing and antioxidant treatment, and driven by polar and non-

polar soluble constituents. Toxicol Lett 238(2):72–82, PMID: 26160521, https://doi.org/10.1016/j.toxlet.2015.07.002.

Behndig AF, Mudway IS, Brown JL, Stenfors N, Helleday R, Duggan ST, et al. 2006. Airway antioxidant and inflammatory responses to diesel exhaust exposure in healthy humans. Eur Respir J 27(2):359–365, PMID: 16452593, https://doi.org/10.1183/09031936.06.00136904.

Bräuner EV, Møller P, Barregard L, Dragsted LO, Glasius M, Wåhlin P, et al. 2008. Exposure to ambient concentrations of particulate air pollution does not influence vascular function or inflammatory pathways in young healthy individuals. Part Fibre Toxicol 5:13, PMID: 18837984, https://doi.org/10.1186/1743-8977-5-13.

Brook RD, Rajagopalan S, Pope CA 3rd, Brook JR, Bhatnagar A, Diez-Roux AV, et al. 2010. Particulate matter air pollution and cardiovascular disease: An update to the scientific statement from the American Heart Association. Circulation 121(21):2331–2378, PMID: 20458016, https://doi.org/10.1161/CIR.0b013e3181dbece1.

Brown MS, Sarnat SE, DeMuth KA, Brown LAS, Whitlock DR, Brown SW, et al. 2012. Residential proximity to a major roadway is associated with features of asthma control in children. PLoS One 7(5):e37044, PMID: 22615884, https://doi.org/10.1371/journal.pone.0037044.

Calderon-Garciduñas L, Macías-Parra M, Hoffmann HJ, Valencia-Salazar G, Henriquez-Roldan C, Osnaya N, et al. 2009. Immunotoxicity and environment: Immunodysregulation and systemic inflammation in children. Toxicol Pathol 37(2):161–169, PMID: 19171930, https://doi.org/10.1177/0192623308329340.

Calderon-Garciduñas L, Mora-Tiscareño A, Francolira M, Torres-Jardón R, Peña-Cruz B, Palacios-López C, et al. 2013. Exposure to urban air pollution and bone health in clinically healthy six-year-old children. Arh Hig Rada Toksikol 64(1):23–34, PMID: 23612523, https://doi.org/10.2478/10004-1254-64-2013-2219.

Chuang KJ, Yan YH, Chiu SY, Cheng T-J. 2011. Long-term air pollution exposure and risk factors for cardiovascular diseases among the elderly in Taiwan. Occup Environ Med 68(1):64–68, PMID: 20833756, https://doi.org/10.1136/oem.2009.052704.

Chung F. 2001. Anti-inflammatory cytokines in asthma and allergy: Interleukin-10, interleukin-12, interferon-gamma. Mediators Inflamm 10(2):51–59, PMID: 11405550, https://doi.org/10.1080/09629350120054518.

Clark NA, Demers PA, Karr CJ, Koehoorn M, Lencar C, Tamburic L, et al. 2010. Effect of early life exposure to air pollution on development of childhood asthma. Environ Health Perspect 118(2):284–290, PMID: 20123607, https://doi.org/10.1289/ehp.0900916.

Dobreva ZG, Miteva LD, Stanilova SA. 2009. The inhibition of JNK and p38 MAPKs downregulates IL-10 and differentially affects c-Jun gene expression in human monocytes. Immunopharmacol Immunotoxicol 31(2):195–201, PMID: 19235539, https://doi.org/10.1080/08923970802626276.

Dobreva ZG, Kostadinova GS, Popov BN, Petkov GS, Stanilova SA. 2015. Proinflammatory and anti-inflammatory cytokines in adolescents from southeast Bulgarian cities with different levels of air pollution. Toxicol Ind Health 31(12):1210–1217, PMID: 23771874, https://doi.org/10.1177/0748233713491812.

Eneroth K, Johansson C, Bellander T. 2006. EXPOSURE - Comparison between measurements and calculations based on exposure modelling. Report lvf 2006:12. http://www.Slb.Nu/slb/rapporter/pdf/lvf2006_12.Pdf [accessed 11 June 2015].

Esposito S, Tenconi R, Lelii M, Preti V, Nazzari E, Consolo S, et al. 2014. Possible molecular mechanisms linking air pollution and asthma in children. BMC Pulm Med 14:31, PMID: 24581224, https://doi.org/10.1186/1471-2466-14-31.

Forbes LJ, Patel MD, Rudnicka AR, Cook DG, Bush T, Stedman JR, et al. 2009. Chronic exposure to outdoor air pollution and markers of systemic inflammation. Epidemiology 20(2):245–253, PMID: 19234416, https://doi.org/10.1097/EDE.0b013e318190ea3f.

Gehring U, Wijga AH, Hoek G, Bellander T, Berdel D, Brüske I, et al. 2015. Exposure to air pollution and development of asthma and rhinoconjunctivitis throughout childhood and adolescence: A population-based birth cohort study. Lancet Resp Med 3(12):933–942, PMID: 27057569, https://doi.org/10.1016/S2213-2600(15)00426-9.

Gruzieva O, Bellander T, Eneroth K, Kull I, Melén E, Nordling E, et al. 2012. Traffic-related air pollution and development of allergic sensitization in children during the first 8 years of life. J Allergy Clin Immunol 129(1):240–246, PMID: 22104609, https://doi.org/10.1016/j.jaci.2011.11.001.

Gruzieva O, Xu C-J, Breton CV, Annesi-Maesano I, Antó JM, Auffray C, et al. 2017. Epigenome-wide meta-analysis of methylation in children related to prenatal NO$_2$ air pollution exposure. Environ Health Perspect 125(1):104–110, PMID: 27448387, https://doi.org/10.1289/EHP36.

Hajat A, Allison M, Diez-Roux AV, Jenny NS, Jorgensen NW, Szpiro AA, et al. 2015. Long-term exposure to air pollution and markers of inflammation, coagulation, and endothelial activation: A repeat-measures analysis in the multi-ethnic study of atherosclerosis (mesa). Epidemiology 26(3):310–320, PMID: 25710246, https://doi.org/10.1097/EDE.0000000000000267.

Hoffmann B, Moebus S, Dragano N, Stang A, Möhlenkamp S, Schmermund A, et al. 2009. Chronic residential exposure to particulate matter air pollution and

systemic inflammatory markers. Environ Health Perspect 117(18):1302–1308, PMID: 19672412, https://doi.org/10.1289/ehp.0800362.

Kishimoto T. 2006. Interleukin-6: Discovery of a pleiotropic cytokine. Arthritis Res Ther 8 (suppl 2):S2, PMID: 16899106, https://doi.org/10.1186/ar1916.

Klümper C, Krämer U, Lehmann I, von Berg A, Berdel D, Herberth G, et al. 2015. Air pollution and cytokine responsiveness in asthmatic and non-asthmatic children. Environ Res 138:381–390, PMID: 25769127, https://doi.org/10.1016/j.envres.2015.02.034.

Kohli A, Garcia MA, Miller RL, Maher C, Humblet O, Hammond SK, et al. 2012. Secondhand smoke in combination with ambient air pollution exposure is associated with increasedx CpG methylation and decreased expression of IFN-γ in T effector cells and Foxp3 in T regulatory cells in children. Clin Epigenetics 4(1):17, PMID: 23009259, https://doi.org/10.1186/1868-7083-4-17.

Kongerud J, Madden MC, Hazucha M, Peden D. 2006. Nasal responses in asthmatic and nonasthmatic subjects following exposure to diesel exhaust particles. Inhal Toxicol 18(9):589–594, PMID: 16864550, https://doi.org/10.1080/08958370600743027.

Korek M, Johansson C, Svensson N, Lind T, Beelen R, Hoek G, et al. 2016. Can dispersion modeling of air pollution be improved by land-use regression? An example from Stockholm, Sweden. J Expo Sci Environ Epidemiol Aug 3. [Epub ahead of print], PMID: 27485990, https://doi.org/10.1038/jes.2016.40.

Larsson N, Brown J, Stenfors N, Wilson S, Mudway IS, Pourazar J, et al. 2013. Airway inflammatory responses to diesel exhaust in allergic rhinitics. Inhal Toxicol 25(3):160–167, PMID: 23421487, https://doi.org/10.3109/08958378.2013.765932.

Leem JH, Kim JH, Lee KH, Hong YC, Lee KH, Kang D, et al. 2005. Asthma attack associated with oxidative stress by exposure to ETS and PAH. J Asthma 42(6):463–467, PMID: 16293541, https://doi.org/10.1080/02770900500200733.

Liu Q, Baumgartner J, Zhang Y, Liu Y, Sun Y, Zhang M. 2014. Oxidative potential and inflammatory impacts of source apportioned ambient air pollution in Beijing. Environ Sci Technol 48(21):12920–12929, PMID: 25279798, https://doi.org/10.1021/es5029876.

Ljungman P, Bellander T, Schneider A, Breitner S, Forastiere F, Hampel R, et al. 2009. Modification of the interleukin-6 response to air pollution by interleukin-6 and fibrinogen polymorphisms. Environ Health Perspect 117(9):1373–1379, PMID: 19750100, https://doi.org/10.1289/ehp.0800370.

Melén E, Granell R, Kogevinas M, Strachan D, Gonzalez JR, Wjst M, et al. 2013. Genome-wide association study of body mass index in 23 000 individuals with and without asthma. Clin Exp Allergy 43(4):463–474, PMID: 23517042, https://doi.org/10.1111/cea.12054.

Minelli C, Wei I, Sagoo G, Jarvis D, Shaheen S, Burney P. 2011. Interactive effects of antioxidant genes and air pollution on respiratory function and airway disease: A HuGE review. Am J Epidemiol 173(6):603–620, PMID: 21343247, https://doi.org/10.1093/aje/kwq403.

Moffatt MF, Gut IG, Demenais F, Strachan DP, Bouzigon E, Heath S, et al. 2010. A large-scale, consortium-based genomewide association study of asthma. N Engl J Med 363(13):1211–1221, PMID: 20860503, https://doi.org/10.1056/NEJMoa0906312.

Mostafavi N, Vlaanderen J, Chadeau-Hyam M, Beelen R, Modig L, Palli D, et al. 2015. Inflammatory markers in relation to long-term air pollution. Environ Int 81:1–7, PMID: 25898227, https://doi.org/10.1016/j.envint.2015.04.003.

Nakane H. 2012. Translocation of particles deposited in the respiratory system: A systematic review and statistical analysis. Environ Health Prev Med 17(4):263–274, PMID: 22101916, https://doi.org/10.1007/s12199-011-0252-8.

Nazariah SS, Juliana J, Abdah MA. 2013. Interleukin-6 via sputum induction as biomarker of inflammation for indoor particulate matter among primary school children in Klang Valley, Malaysia. Glob J Health Sci 5(4):93–105, PMID: 23777726, https://doi.org/10.5539/gjhs.v5n4p93.

Nordling E, Berglind N, Melén E, Emenius G, Hallberg J, Nyberg F, et al. 2008. Traffic-related air pollution and childhood respiratory symptoms, function and allergies. Epidemiology 19(3):401–408, PMID: 18379426, https://doi.org/10.1097/EDE.0b013e31816a1ce3.

Panasevich S, Leander K, Rosenlund M, Ljungman P, Bellander T, de Faire U, et al. 2009. Associations of long- and short-term air pollution exposure with markers of inflammation and coagulation in a population sample. Occup Environ Med 66(11):747–753, PMID: 19687019, https://doi.org/10.1136/oem.2008.043471.

Panasevich S, Leander K, Ljungman P, Bellander T, de Faire U, Pershagen G, et al. 2013. Interaction between air pollution exposure and genes in relation to levels of inflammatory markers and risk of myocardial infarction. BMJ open 3(9):e003058, PMID: 24056475, https://doi.org/10.1136/bmjopen-2013-003058.

Pedroza M, Schneider DJ, Karmouty-Quintana H, Coote J, Shaw S, Corrigan R, et al. 2011. Interleukin-6 contributes to inflammation and remodeling in a model of adenosine mediated lung injury. PLoS One 6(7):e22667, PMID: 21799929, https://doi.org/10.1371/journal.pone.0022667.

Perneger TV. 1998. What's wrong with Bonferroni adjustments. BMJ 316(7139):1236–1238, PMID: 9553006.

Rincon M, Irvin CG. 2012. Role of IL-6 in asthma and other inflammatory pulmonary diseases. Int J Biol Sci 8(9):1281–1290, PMID: 23136556, https://doi.org/10.7150/ijbs.4874.

Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, et al. 2015. Limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res 43(7):e47, PMID: 25605792, https://doi.org/10.1093/nar/gkv007.

Rückerl R, Greven S, Ljungman P, Aalto P, Antoniades C, Bellander T, et al. 2007. Air pollution and inflammation (interleukin-6, C-reactive protein, fibrinogen) in myocardial infarction survivors. Environ Health Perspect 115(7):1072–1080, PMID: 17637925, https://doi.org/10.1289/ehp.10021.

Saito Y, Azuma A, Kudo S, Takizawa H, Sugawara I. 2002. Long-term inhalation of diesel exhaust affects cytokine expression in murine lung tissues: Comparison between low- and high-dose diesel exhaust exposure. Exp Lung Res 28(6):493–506, PMID: 12217215, https://doi.org/10.1080/01902140290096764.

Schultz ES, Hallberg J, Bellander T, Bergström A, Bottai M, Chiesa F, et al. 2016. Early-life exposure to traffic-related air pollution and lung function in adolescence. Am J Respir Crit Care Med 193(2):171–177, PMID: 26397124, https://doi.org/10.1164/rccm.201505-0928OC.

Shang Y, Fan L, Feng J, Lv S, Wu M, Li B, et al. 2013. Genotoxic and inflammatory effects of organic extracts from traffic-related particulate matter in human lung epithelial a549 cells: The role of quinones. Toxicol In Vitro 27(2):922–931, PMID: 23333790, https://doi.org/10.1016/j.tiv.2013.01.008.

Shi TY, Zhu ML, He J, Wang MY, Li QX, Zhou XY, et al. 2013. Polymorphisms of the interleukin 6 gene contribute to cervical cancer susceptibility in eastern Chinese women. Hum Genet 132(3):301–312, PMID: 23180271, https://doi.org/10.1007/s00439-012-1245-4.

Sonnenschein-van der Voort AM, de Kluizenaar Y, Jaddoe VW, Gabriele C, Raat H, Moll HA, et al. 2012. Air pollution, fetal and infant tobacco smoke exposure, and wheezing in preschool children: A population-based prospective birth cohort. Environ Health 11:91, PMID: 23231783, https://doi.org/10.1186/1476-069X-11-91.

Thacher JD, Gruzieva O, Pershagen G, Neuman Å, Wickman M, Kull I, et al. 2014. Pre- and postnatal exposure to parental smoking and allergic disease through adolescence. Pediatrics 134(3):428–434, PMID: 25136039, https://doi.org/10.1542/peds.2014-0427.

Thompson AM, Zanobetti A, Silverman F, Schwartz J, Coull B, Urch B, et al. 2010. Baseline repeated measures from controlled human exposure studies: Associations between ambient air pollution exposure and the systemic inflammatory biomarkers IL-6 and fibrinogen. Environ Health Perspect 118(1):120–124, PMID: 20056584, https://doi.org/10.1289/ehp.0900550.

Totlandsdal AI, Cassee FR, Schwarze P, Refsnes M, Låg M. 2010. Diesel exhaust particles induce CYP1A1 and pro-inflammatory responses via differential pathways in human bronchial epithelial cells. Part Fibre Toxicol 7:41, PMID: 21162728, https://doi.org/10.1186/1743-8977-7-41.

Tsai DH, Amyai N, Marques-Vidal P, Wang JL, Riediker M, Mooser V, et al. 2012. Effects of particulate matter on inflammatory markers in the general adult population. Part Fibre Toxicol 9:24, PMID: 22769230, https://doi.org/10.1186/1743-8977-9-24.

Velásquez IM, Frumento P, Johansson K, Berglund A, de Faire U, Leander K, et al. 2014. Association of interleukin 8 with myocardial infarction: Results from the Stockholm Heart Epidemiology Program. Int J Cardiol 172(1):173–178, PMID: 24462138, https://doi.org/10.1016/j.ijcard.2013.12.170.

Wickman M, Kull I, Pershagen G, Nordvall SL. 2002. The BAMSE project: Presentation of a prospective longitudinal birth cohort study. Pediatr Allergy Immunol 13 (suppl 15):11–13, PMID: 12688617, https://doi.org/10.1034/j.1399-3038.13.s.15.10.x.

Wouters EF, Reynaert NL, Dentener MA, Vernooy JH. 2009. Systemic and local inflammation in asthma and chronic obstructive pulmonary disease: Is there a connection?. Proc Am Thorac Soc 6(8):638–647, PMID: 20008867, https://doi.org/10.1513/pats.200907-073DP.

Zhou F, Cheng L, Qiu L, Wang MY, Li J, Sun MH, et al. 2016. Associations of potentially functional variants in il-6, jaks and stat3 with gastric cancer risk in an eastern Chinese population. Oncotarget 7(19):28112–28123, PMID: 27049718, https://doi.org/10.18632/oncotarget.8492.

Zuurbier M, Hoek G, Oldenwening M, Meliefste K, Krop E, van den Hazel P, et al. 2011. In-traffic air pollution exposure and CC16, blood coagulation, and inflammation markers in healthy adults. Environ Health Perspect 119(10):1384–1389, PMID: 21665568, https://doi.org/10.1289/ehp.1003151.