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Tumour necrosis factor α polymorphism (TNF-308α G/A) in association with asthma related phenotypes and air pollutants among children in KwaZulu-Natal

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Abstract

Background: The study of gene-environment interactions enables us to further understand the pathogenesis of asthma and inflammation. The TNF-α gene has been associated with airway pathology in asthma but there is limited information in relation to pollutant exposure and the TNF-α 308G/A polymorphism.

Objective: To determine the risk conferred by the TNF-α 308(G/A) polymorphism on respiratory outcome and to evaluate whether the association between exposure to ambient air pollutants such as SO_2, NO_2, NO, and PM_{10} and variation in lung function measures is modified by genotype.

Methods: The sample comprised 129 African children (between 9-11 years old). A questionnaire based on guidelines from the British Medical Research Council and the American Thoracic Society was administered to all caregivers to evaluate the prevalence of respiratory symptoms. Atopy was evaluated by skin prick testing. Bihourly measures of lung function (spirometry) were collected at school five days per week over three week periods in each of four seasons (2004-2005) using digital hand-held devices. During each of the four intensive 3-week phases, gaseous air pollutant concentrations were monitored continuously. Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-PFLP) analysis was used to detect the TNF-α 308 genotype and plasma TNF-α levels were measured using the human TNF-α Max Standard™ Enzyme-linked immuno-absorbent assay (ELISA) kit.

Results: The TNF-α variant A allele was common among this sample of African children (40% with an allelic frequency of 0.24). There was no significant association with the TNF-α G/A polymorphism and any respiratory linked phenotype, nor cytokine levels. However, when exposure to pollutants were analyzed with genotypic and phenotypic data, we found relatively modest interaction effects for the TNF-α 308 genotype. GEE models showed that children with the TNF-α 308 A allele had increased deterioration of lung function post pollution exposure to SO_2 [β=2.62, CI:0.51-4.71, p=0.02 and p_{int}=0.03] and NO [β=3.28, CI:0.68-5.89, p=0.01, p_{int}=0.03].

Conclusion: The TNF-α 308 (G/A) polymorphism may be associated with increased pollutant-associated effects on FEV_1 intraday variability for both SO_2 and NO. The A allele may increase susceptibility to the adverse effects of air pollutants.

Keywords: TNF-308α G/A, respiratory phenotypes, air pollution, asthma, biomarker

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Introduction

Gene-environment studies have focused on functional single nucleotide polymorphisms in candidate genes that are predicted to play a role in environmental stressors and mediate the effects of exposure. To this end, the study of gene-environment interactions enables us to further understand the pathogenesis of allergic disease and inflammation.[1]

TNF-α is a 157 amino acid pro-inflammatory cytokine which activates the immune system in response to cancer, infection, exposure to endotoxins, or other products of bacterial, viral, parasitic or inflammatory origin.[2] TNF-α has been implicated in many aspects of airway pathology in asthma.[3] Data suggest that TNF-α plays a role in the initiation of allergic asthmatic airway inflammation and the generation of airway hyper-reactivity.[4] Pollution exposure studies have found increased amounts of proinflammatory cytokines, chemokines and adhesion molecules in bronchial lavage fluid and nasal washes[5] and in serum.[2] *In vitro* studies have demonstrated a direct effect of pollutants on the expression of these markers. O_3, SO_2 and diesel exhaust particles (DEPs) may cause the release of soluble intercellular adhesion molecules and proinflammatory cytokines.[5]

A single nucleotide substitution of the G allele with an A allele polymorphism at −308 nucleotides upstream from the transcription initiation site in the TNF-α promoter is associated with elevated TNF-α levels.[6] The TNF-α 308 A allele is associated with increased levels of TNF-α in plasma and bronchoalveolar lavage fluid from asthmatic airways.[7] The most recent meta-analysis on TNF-α 308 found that carriers of the variant A allele had an increased risk of asthma compared with those who were homozygous for the GG allele (OR=1.40, 95% CI:1.13-1.68). Significantly elevated risks were associated with the A allele in Asians (OR=1.53, 95% CI=1.17-2.01) but not Caucasians (OR=1.06, 95% CI=0.75-1.50).[3] However, data from African populations for such analyses in association with respiratory linked outcomes are limited.

In a South African population of black African children, the frequency of the TNF-α 308(G/A) polymorphism and the risk conferred on respiratory outcomes was determined. In addition, we evaluated whether the association between exposure to ambient air pollutants such as SO_2, NO_2, NO, and PM_{10} and variation in lung function measures was modified by genotype.

Methods

Study Population

This study reports on a sample of 129 indigenous African children (between 9-11 years old) from 7 primary schools recruited for a larger study from south and north Durban in South Africa. At each of the 7 schools, two 4th grade classrooms were randomly prioritized as classroom 1 and 2 and children were randomly selected from these classrooms. A total of 317 children participated in the larger study. For this study, we used a subset of the participants, all those of African descent (n=148), in order to eliminate the effect of ethnicity on genetic markers. Viable "banked" blood samples were used in the analysis, further restricting our sample to 129.

Details of the study sampling strategy have been presented elsewhere.[8] The south Durban region, recognized as one of the most highly industrialized and most heavily polluted areas in Southern Africa,[9] includes extensive residential areas. This study was approved by the University of KwaZulu-Natal and the Durban University of Technology's Ethics Committees, and parental consent for genetic analyses was obtained for all children.

Symptom and demographic data

A questionnaire was administered to all caregivers, which included components addressing demographic information, and respiratory and other relevant symptoms using standardized validated questions from sources such as the British Medical Research Council and the American Thoracic Society. Characterization of asthma severity (any asthma and persistent asthma) was reported previously.[8] Briefly, questions addressing the presence and severity of asthma included information concerning wheezing, coughing, chest tightness, shortness of breath, activity limitations, and medication use, health services utilization, quality of life measures, perinatal history, place of birth and residential history, exercise, viral respiratory infections, exposure to cigarette smoke and pre-existing medical conditions. Asthma severity was categorized in two ways: asthma of any severity and persistent asthma. A child was considered to have asthma of any severity if any of the following were true: three or more non-exercise-related symptoms (e.g. cough, wheeze, chest tightness), exercise-induced wheeze or cough reported at a frequency of three or more times during the previous year, doctor diagnosed asthma, reactive airway disease and/or asthmatic bronchitis or doctor prescribed medication taken in the previous year. A child was considered to have "persistent asthma" if, firstly, the child met the diagnostic criteria for asthma of any severity and secondly, any of the following were true: any daytime symptom (cough, wheeze, exercise induced cough and wheeze and chest tightness) was reported as being present more than two times per week, sleep disturbances due to cough, wheeze, shortness of breath or chest tightness reported more than two times per month, and/or the daily use of doctor prescribed medication.

Baseline spirometry was performed by experienced technologists using the American Thoracic Society criteria using the Jaeger Flowscreen.[10] Spirometers were calibrated at least twice a day with a 3 L syringe. Children without a baseline obstructive pattern underwent methacholine nonspecific challenge testing by trained technologists according to an abbreviated protocol used in epidemiological surveys.[10] Participants with an obstructive pattern at baseline (FEV_1/FVC <0.75) were administered an inhaled bronchodilator and had testing repeated. Bronchial Airway hyper-reactivity (BHR) was categorized as marked (PC20, dose of methacholine causing a 20% fall in baseline FEV_1, ≤4 mg/ml), probable (4 mg/ml<PC20≤8 mg/ml), borderline/possible (8 mg/ml<PC20≤16 mg/ml), and no hyper-reactivity (PC20 ≥16 mg/ml). Marked, probable, and possible AHR were categorized as "any evidence of bronchial hyper-reactivity."

Atopy was defined as a positive response to one or more of the following antigens: mixed cockroach, mixed dust mite, mold mix (Aspergillus, Cladosporium, and Penicillium), cat, dog, mouse, rat, and grass by skin prick testing, with histamine as a positive control and saline as a negative control. A greater than 3-mm difference in mean diameter between allergen and control wheal was considered positive.

Bihourly measures of pulmonary function

A central aspect of the health data collection was bihourly measures of lung function (spirometry) collected at school five days per week over three week periods in each of four seasons using a digital hand-held device: the AirWatch® (iMetrikus, Carlsbad, California, USA) brand airway monitor. All schools were studied simultaneously in the same calendar periods. On each of the five schooldays, during the week, participants were asked to perform a session of three consecutive forced expiratory maneuvers every one and a half to two hours (four times per 5.5 hour school day: at approximately 08h00, 09h45, 11h30 and 13h20). Results of repeated expiratory maneuvers over a period of 12 months were digitally stored in each AirWatch. The highest FEV_1 from a valid expiratory maneuver for each of the four daily sessions was used in data analyses. An expiratory maneuver was considered valid if the FEV_1 result 1) was between 30 and 120% of each child's personal best as defined by that child's highest recorded FEV_1 during baseline spirometry (performed by experienced technicians using the American Thoracic Society criteria) and methacholine challenge testing, and, 2) came from an expiratory maneuver that was recorded by the AirWatch device as "error-free."

Environmental monitoring of ambient pollutants

During each of the four intensive 3-week phases, gaseous air pollutant concentrations were monitored continuously: NO_2 and NO were sampled at seven monitoring sites using continuous gas-phase chemiluminescence detection; SO_2 was monitored continuously at 16 sites, including all seven schools, using ultraviolet fluorescence spectrometry. PM_{10} was monitored gravimetrically at 12 sites: at each school using 24-hr integrated measurements, and at five additional sites using TEOMs. Each pollutant was sampled using standard reference methods and protocols. Details of these measurements and the quality assurance program are reported elsewhere.[11]

Genotyping and analysis of plasma TNF-α concentration

All genotyping assays were conducted by a researcher who was blind to child ID and disease status. Genomic DNA was extracted using a PUREGENE DNA isolation kit (GENTRA). The Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-PFLP) method was used to detect the TNF-α 308 genotype.[12] Plasma TNF-α levels were measured using the human TNF-α Max Standard™ Enzyme-linked immuno-absorbent assay (ELISA) kit (Biolegend) following the manufacturer's instructions. Absorbance was measured at 450nm using the Bio-Tek μQuant ELISA plate reader. Plasma concentrations of TNF-α were extrapolated from a standard curve.

Statistical Analysis

Initial descriptive analysis was followed by bivariate testing. TNF was dichotomized into two groups based on the absence or presence of the polymorphic allele (wild type homozygous, heterozygote and homozygous variant). Differences in genotype frequency between cases and non-cases were assessed by the chi-squared test. Associations of genotype with asthma, atopy and BHR were examined using multivariate logistic regression models using race, gender and exposure to environmental tobacco smoke as covariates. Linear regression models were fitted using generalized estimating equations (GEEs, assuming normal distribution with identity link) using PROC GENMOD for SAS to accommodate the correlation structure arising from repeated measurements on the same individual.

Within-day variability for FEV_1 was defined as 100 x (the maximum best FEV_1- minimum best FEV_1)/maximum best FEV_1 where the "best FEV_1" is the highest valid value for the specific time of day (08h00, 09h45, and 11h30, 13h20), thus providing a single summary lung function measurement per child, per day. Effect modification was examined by including genotype and pollutant product terms in the models. The gene-environment-interaction was assessed for associations of exposure to SO_2, NO, NO_2 and PM_{10} with FEV_1 intraday variability, using the TNF-α 308G/A genotype as an effect modifier. Lag effects were modeled to account for both acute and prior exposure effects, and included lags of 1 to 2 days as well as the a 5-day average. The percent change in within-day variability in FEV_1 were estimated for an increase of one interquartile range in each pollutant (NO_2: 8.19 ppb, NO: 29.7 ppb, PM_{10}: 29.4 ug m^{-3} and SO_2: 9.8 ppb). The interquartile range was calculated as the 75^{th}-25^{th} percentile value concentrations, using all of the concentration measurements obtained in the study. An adverse effect in lung function would be denoted by an increase in the estimate for intraday variability in FEV_1. SAS (Version 9.1) and STATA (version 9, College Station, TX, USA) were used for analyses.

Results

Demographic, phenotypic and genotypic characteristics of the population sample are summarized in **Table 1**. The average age was 10.3 years and 71% of children were female. Half of the children in the sample were exposed to tobacco smoke in their households. Approximately 28% of all participants reported symptoms of asthma, while nearly a third of the population was atopic (27.4%). Approximately 40% of the study population carried the TNF-α variant (GA/AA). The TNF-α 308AA genotype was relatively rare among participants (8%). Genotype frequencies did not deviate from Hardy Weinberg equilibrium (p=0.29). Bivariate and multivariate logistic regression modelling showed no statistically significant association between the TNF-α 308 G/A promoter polymorphism with asthma related phenotypes such as bronchial hyper-responsiveness and atopy (**Table 2**). TNF-α levels stratified by genotype and respiratory phenotype are presented in Figure 1. There were no significant differences in TNF-α levels between the wild type and polymorphic

Table 1. Demographic, phenotypic and genotypic characteristics of study population (n=104)

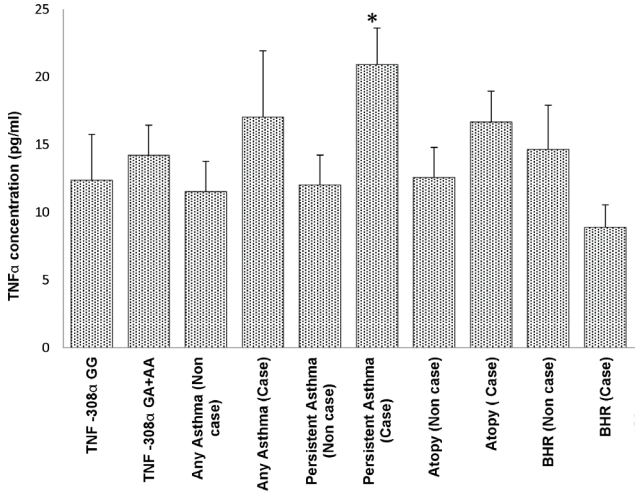
Variable	n=104 (%)
Age (mean, SD)	10.3 (2.4)
Female	71 (68.3)
Prevalence of respiratory outcomes	
Any Asthma	35 (38.9)
Persistent Asthma	14 (15.6)
Atopy	26 (27.4)
Bronchial Hyper responsiveness (BHR)	15 (18.8)
Environmental Exposures	
Exposure to Environmental Tobacco Smoke	48 (50)
Genotyping	
TNF- 308 α wild type (GG)	62 (59.6)
TNF- 308 α heterozygote (GA)	34 (32.7)
TNF- 308 α homozygote (AA)	8 (7.7)
TNF- 308 α variant (GA+AA)	42 (40.3)

Table 2. Association of TNF-308α gene polymorphisms with respiratory outcome

Respiratory outcome		TNF-308α GG	TNF-308α GA+AA
Any Asthma	Non case	31 (57.4)	24 (66.7)
	Case	13 (29.5)	12 (33.3)
	*OR (95% Cl)	1.00	0.89 (0.35,2.26)
Persistent Asthma	Non case	44 (81.5)	32 (88.9)
	Case	10 (18.5)	4 (11.1)
	OR (95% Cl)	1.00	0.46 (0.12,1.69)
Atopy	Non case	44 (74.6)	25 (69.4)
	Case	15 (25.4)	11 (30.6)
	OR (95% Cl)		1.31 (0.52,3.33)
BHR	Non case	40 (80)	25 (83.3)
	Case	10 (20)	5 (16.67)
	OR (95% Cl)	1.00	0.84 (0.25,2.79)

*Logistic regression models adjusted for age and gender

Figure 1. Mean TNFα concentration (including standard deviation) stratified by genotype and respiratory outcome (n=104).



genotype. TNF-α levels were generally higher in atopic compared with non-atopic children. Children with persistent asthma showed significantly higher TNF-α levels compared with those with no persistent asthma (20.93pg/ml vs 12.04pg/ml, p=0.03).

Table 3 summarizes 24–hour average pollutant levels in Durban. PM_{10} levels during the study period were high relative to WHO guidelines [WHO, 2006]; the 24-hr guideline of 50 μg/m^3 was frequently exceeded. SO_2 levels varied widely across the study area, and the highest concentrations occurred in south Durban, reflecting the location of emitting industries. The WHO 24-hr guideline value of 8 ppb was frequently exceeded for SO_2. NO_2 levels were highest in the city center and reflected traffic emissions. WHO guideline values for NO_2 exist on an annual average (21 ppb) and 1-hour (106 ppb) basis, but not for 24-hour averages. During the study period, the annual average level was similar to guideline value, and 1-hour concentrations at the city center site occasionally exceeded the 1-hour guideline value.

GEE models were used to assess the relationship between SO_2, NO, NO_2 and PM_{10} exposure and changes in lung function (FEV_1 and PF) using the TNF-α308 polymorphic genotype as an effect modifier (**Table 4**). Increased intraday variability estimates indicated adverse lung function. At 2 days post exposure (lag 2) there was a significant association between lung function (FEV_1 intraday variability) and PM_{10} in GG individuals (β= 1.35, CI:0.12,2.58, p=0.03). The effect estimates of SO_2 for children who were carriers of the A allele were generally higher, reaching a significant gene pollutant interaction with intraday variability in FEV_1 at lag 2 (β= 2.62, CI: 0.51-4.71, p=0.02. p_{int}=0.03) indicating a decline in lung function post-pollution exposure in children carrying the A allele. Similarly the effect of SO_2 exposure was significantly modified by genotype using intraday variability in PEF as an outcome. Children with the TNF-α 308AA/AG genotype had a higher estimate compared to children carrying the wild type (p_{int}=0.04). In addition, children who were carriers of the A allele showed statistically significant adverse effect modifications when exposed to increasing levels of NO (β=3.28, CI:0.68-5.89, p=0.01, p_{int}=0.03) at lag 2. In spite of a few conflicting results (i.e., higher exposure associated with decreased variability), effect estimates generally showed an adverse respiratory effect of increased pollutant exposure on lung function measures among children with the TNF-α 308AA/AG genotype.

Discussion

Studies from the African continent on the association of the TNF-α -308G/A polymorphism with various diseases has been published but there are limited data on asthma linked with pollutant exposures. The TNF-α 308 variant A allele was common among this sample of African children (40% with an allelic frequency of 0.24). While almost a third of the population reported symptoms of asthma and atopy, there was no significant association with the TNF-α 308G/A polymorphism and any respiratory-linked phenotype, nor was there any association between the TNF-α 308G/A genotype and cytokine levels. However, when exposure to pollutants was

analyzed with genotypic and phenotypic data, we found relatively modest interaction effects for the TNF-α 308 genotype. GEE models showed that children with the TNF-α 308 A allele had increased deterioration of lung function post-pollution exposure to SO_2 [β= 2.62, CI (0.51,4.71) p=0.02 and p_{int}=0.03] and NO [β=3.28, CI:0.68-5.89, p=0.01, p_{int}=0.03].

Our results for the frequency of the variant A allele was higher (40%) compared to a South African (20%) and Zimbabwean population (20%) but compared well with frequencies reported from European populations (39%).[3] Very low frequencies have been reported for Chinese (8.7%) and Korean (7.6%) individuals.[3,13] There were no significant differences in TNF-α level between the wild type and polymorphic genotype. Although many studies have examined the functional consequence of the TNF-α308 G/A SNP on TNFα production, the results remain controversial. Some have reported higher TNF-α production by G/A donors compared to G/G donors,[13] while others studies have reported no significant effect.[3,14] Although we did not find an association between the TNF-α 308G/A polymorphism and respiratory outcomes, we did detect significantly elevated TNF-α levels in children with persistent asthma. Previous data has shown an association between asthma (severity and susceptibility) and the polymorphic TNF-α 308 allele.[15,16] This is biologically plausible as the TNF-α gene increases airway smooth muscle cell contractility and IL-5 secretion. There are also data to implicate TNF-α in airway remodeling and fibrosis.[4] In addition, it has been suggested that association of the TNF-α 308 A allele with asthma reflects linkage disequilibrium with genes influencing a specific immune response.[17]

Primary sources of nitrogen oxides emissions in this study include motor vehicles on the various motorways which converge in the South Durban basin.[8] In addition, nitrogen oxides react with other pollutants to form ground-level ozone which may irritate the nose and throat, especially in people with asthma, and appears to increase susceptibility to respiratory infections.[18] In the Durban South industrial basin, an oil refinery, a paper producer and sugar refinery are responsible for 80% of the SO_2 pollution load.[19] SO_2 causes heightened sensitivity to allergens that commonly trigger asthma attacks, narrowing of the airways and breathing difficulties, and causes wheezing, chest tightness and shortness of breath even among healthy people who do not have asthma.[18]

Few studies have addressed interactions between air pollutants and TNF-α 308 in relation to respiratory health. The South Durban Health Study provided detailed exposure data for investigating the effect of genotype on the association between air pollution and respiratory health. Generally, the effect estimates showed the adverse effects of increased pollutant exposure on lung function among those carrying the A allele (**Table 4**). The A allele has been associated with higher levels of

Table 3. Summary of ambient pollutant levels (24 hr averages) in Durban during 2004-2005.

Pollutant	Mean (SD)	Range
PM_{10} (μg/m³)	86.8 (1.1)	28.1-266.6
SO_2 (ppb)	5.8 (0.2)	0-40.8
NO_2 (ppb)	22.1 (0.2)	7.5-38.1
NO (ppb)	53.4 (0.8)	1.3-91.7

Table 4. Effect of pollutant exposure stratified by TNF gene polymorphisms. Percent change[1] in intraday variability[2] of FEV_1 and Intraday Variability of PEF[3], associated with ambient levels[6] of pollutants (SO_2 and PM_{10})from single pollutant linear regression models using generalized estimating equations (GEE).

Lung Function Outcome	Genotype	Lag	SO_2 β (CI)	PM_{10} β (CI)	NO_2 β (CI)	NO β (CI)
Intraday Variability Fev_1	TNFAA/GA	Lag 1	2.28 (-0.28,4.85)	0.4 (-0.93,1.73)	1.85 (1.35-3.36)	-2.44 (-5.28,0.41)
	TNF GG		0.81(-1.29,2.90)	0.06 (-0.88,1.00)	1.38 (-1.21,3.98)	-0.63 (-2.29,1.04)
	TNFAA/GA	Lag 2	2.62 (0.51,4.71)*†	0.88 (-1.31,3.07)	-1.86 (-4.34,0.62)	3.28 (0.68,5.89)*†
	TNF GG		0.24 (-1.17,1.65)	1.35 (0.12,2.58)*	0.7 (-1.21,2.61)	0.33 (-1.59,2.25)
	TNFAA/GA	5 days	3.5 (-1.63,8.62)	1.91 (-1.70,5.52)	-1.4 (-5.01,2.22)	-1.38 (-7.60,4.84)
	TNF GG		1.02 (-3.67,5.70)	2.02 (-0.47,4.50)	2.39 (-0.79,5.57)	1.96 (-2.37,6.28)
Intraday Variability PEF	TNFAA/GA	Lag 1	1 (-1.52,3.52)	-0.05 (-0.99,0.9)	-1 (-3.01,1.02)	-0.12 (-2.57,2.32)
	TNF GG		0.14 (-1.58,1.86)	-0.15 (-0.81,0.51)	0.54 (-0.71,1.78)	0.11 (-1.11,1.32)
	TNFAA/GA	Lag 2	1.54 (-0.44,3.52)†	-0.64 (-1.92,0.65)	0.24 (-2.81,3.29)	-0.59 (-3.45,2.27)
	TNF GG		-0.43 (-2.06,1.19)	0.25 (-0.58,1.09)	0.23 (-1.14,1.59)	-0.47 (-1.87,0.92)
	TNFAA/GA	5 days	3.4 (-1.59,8.39)	-0.03 (-2.51,2.45)	0.6 (-4.62,5.82)	2.44 (-3.38,8.27)
	TNF GG		2.03 (-1.97,6.04)	0.73 (-1.29,2.74)	1.97 (-0.36,4.3)	1.51 (-1.46,4.48)

[1] The percent change value shown is for an increase of one interquartile range in each respective pollutant: NO_2: 8.19 ppb; NO: 29.7 ppb, PM_{10}: 29.4 μg m^{-3}; SO_2: 9.8 ppb

[2] Intraday variability for FEV_1 is defined as : 100 (maximum best FEV_1-minimum best FEV_1)/maximum best FEV_1; where the "best FEV_1" is the highest valid, error-free value for the specific time of day (08h00, 09h45, 11h30, 13h20). An increase in the estimate for intraday FEV_1 is indicative of a negative impact on lung function. PEF is defined analogously.

[3] Pollution levels used in regression models combine measured and imputed values

Covariates in each model: race, school, caregiver smoking, caregiver education, household income, season, interaction between genotype and pollutants; *p-value for genotype-pollutant interaction term ≤ 0.05; †p-value for the change in estimate ≤0.05

TNF-α production in several studies and TNF-α has been found in large amounts in bronchial fluids during asthma attacks.[16] As shown in **Table 4**, 2 pollutant-lag combinations showed a statistically significant pollutant-genotype interaction with greater pollution-associated increases in FEV_1 intraday variability for the TNF-α AA/GA genotype. This polymorphic genotype may be associated with increased pollutant associated effects on FEV_1 intraday variability for both SO_2 and NO. Effect estimates of SO_2 exposure on children who were carriers of the A allele were generally higher and a significant gene pollutant interaction was observed with intraday variability of FEV_1 (β= 2.62, 95% CI (0.51, 4.71), p=0.02 p_{int}=0.03) at lag 2. Similarly, a statistically significant gene environment interaction with NO at 2 days post exposure (β= 3.28, 95%CI (0.68, 5.89), p=0.01, p_{int}=0.03) was reported in children with the TNF-α A allele. This supports the notion that the A allele may increase susceptibility to the adverse effects of air pollutants. Conflicting results in an unexpected direction at lag 2 may be due to the fact that we used a 'single gene single pollutant' model and did not account for the complex mixture of pollutants in ambient air which may modify exposure. In addition, susceptibility to respiratory effects is also likely to be modified by SNPs in other genes encoding inflammatory cytokines and metabolizing enzymes.

This study has several strengths. First, the study population of children exposed to ambient pollutants was confined to defined areas, each area with its own monitoring site, allowing a more precise estimation of exposure. Second, pollutants were analyzed in a systematic manner over the duration of the study, which allowed the correlation between increases in exposure and decreases in lung function measures. A limitation was our small sample size. While larger sample sizes will improve power for gene-environment interaction studies, power is also enhanced by better measures of exposure, characterization of individual exposure and repeated measures over time.[20] In addition, inconsistencies in results may be attributed to the fact that we considered each pollutant effect separately and did not account for the complexity of multi-pollutant exposures.

In conclusion, genetic markers such as the TNF-α 308 A allele may help to identify individuals who are at increased risk of adverse respiratory effects from exposure to air pollution. This study supports the importance of further investigation on these and other genotype variants involved in inflammation and respiratory-linked phenotypes in larger cohorts.

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Conflict of interest

The authors declare no conflict of interest.

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