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ORIGINAL ARTICLE

Early-Lifetime Exposure to Air Pollution and Allergic Sensitization in Children with Asthma

KATHLEEN MORTIMER,[1] ROMAIN NEUGEBAUER,[2] FREDERICK LURMANN,[3] SIANA ALCORN,[3] JOHN BALMES,[4,5] AND IRA TAGER[1]

[1]*Division of Epidemiology, School of Public Health, University of California, Berkeley, California*
[2]*Division of Biostatistics, School of Public Health, University of California, Berkeley, California*
[3] *Sonoma Technology, Inc, Petaluma, California*
[4]*Division of Environmental Health Sciences, School of Public Health, University of California, Berkeley, California*
[5]*School of Medicine, University of California, San Francisco, California*

Observations on the association between exposure to common outdoor air pollutants and allergic sensitization have not been consistent. Little research has been done on the effects of prenatal exposure or the effect among asthmatics. The association between prenatal and early-life exposures and outdoor air pollutants with allergic sensitization was examined within a cohort of 170 children ages 6–11 years with asthma, living in the Central Valley of California. Allergic sensitization was ascertained by skin-prick tests to 14 allergens. Prenatal and early-life exposure to ozone (O_3), nitrogen dioxide (NO_2), carbon monoxide (CO) and particulate matter with a median aerodynamic diameter <10 μm (PM_{10}) was reconstructed for each child. Models were developed for sensitized to (a) any allergen, (b) at least one outdoor allergen, and (c) at least one indoor allergen. In multivariable analyses, higher exposure to CO during pregnancy was associated with an increased risk of sensitization to at least one outdoor allergen. The largest effect was seen for the association between exposure to 8-hour daily maximum CO during pregnancy and sensitization to at least one outdoor allergen. (OR = 1.55 (95% CI: 1.01, 2.37)) per interquartile range (IQR) increase.) Similar effects estimates were seen for 2nd trimester exposure to CO, but these were less precisely estimated (OR = 1.45 (95%CI: 0.90, 2.35)). No significant associations with the pollutants were seen for sensitization to allergens in general or to at least one indoor allergen. Exposure to traffic-related pollutants during pregnancy may increase the risk of sensitization to outdoor allergens among asthmatic children.

Keywords air pollution, prenatal, allergen sensitization, asthma

INTRODUCTION

Laboratory studies in humans and animals have shown that air pollutants can enhance allergic inflammation and induce allergic immune responses (1). While it has been shown that exposure to traffic-related pollutants negatively impacts respiratory health, studies of the effect on allergen sensitization have not been consistent, perhaps due to differences in the populations studied with respect to age distributions, (2) asthma status (3), and exposure assessment (4). Cross-sectional comparisons across communities with different pollution profiles (5) may have residual confounding, while those that rely on assessment of current exposures (4, 6) may not capture a critical exposure period. It is essential to focus on exposures that occur well before the recognition of sensitization.

Intrauterine and early childhood factors influence the development of allergen sensitization and allergy (5) Susceptibility may be influenced by mother's atopic status, gender, birth weight, maternal smoking during pregnancy and daycare attendance (5). Sensitization is extremely low in infancy and increases over time (7). The influence of traffic-related air pollutants during the prenatal period has not been investigated, although there is good reason to suspect a role, given the association between traffic-related pollutants and immune modulation (8). For example, exposure to diesel exhaust particles can induce enhanced IgE responses to aeroantigens, and induce isotype switching to specific IgE isoforms (8–10). People with asthma have higher rates of sensitization and allergy and are more responsive to air pollutants' effects on respiratory health (11); this is especially true in children (12). Therefore, an exploratory evaluation of the effect of prenatal and early-life exposure to traffic-related pollutants on sensitization within a cohort of asthmatic children was performed.

MATERIALS AND METHODS

Data for this study were collected during the Fresno Asthmatic Children's Environment Study (FACES), a longitudinal study of 315 children with asthma in Fresno, California. A separately funded sub-study, FACES-Life-Time Exposure (FACES-LiTE) included 170 members of the cohort for whom sufficient prenatal exposure and allergen sensitization status could be obtained. This report examines the impact of those exposures on the risk of allergic sensitization in the FACES-LiTE cohort.

Children and their families were recruited through community-based advertisements, school nurses and local physicians from November 2000–April 2005. A standardized screening interview was conducted to determine if the

Corresponding author: Kathleen M. Mortimer, Division of Epidemiology, School of Public Health, University of California, Berkeley, CA 94720-7370, USA; E-mail: kmort@berkeley.edu

child met the eligibility criteria. To be eligible, a child must have been 6–11 years old, lived in their current residence for at least 3 months, and had physician-diagnosed asthma that was active (i.e., required medication or had recent symptoms or health care utilization). An extensive, in-person interview included demographic information, the child's medical history, early-life and home characteristics, pulmonary function testing, allergen skin testing and family history of allergy and asthma. Details of the study visits and protocols are described in the on-line repository.

During an in-person visit, parents reported the street address, city and state of all residences at which the mother lived during pregnancy and addresses at which the child lived prior to enrollment in FACES. Each address was geocoded with "EZ Locate Client" v.1.61, by Tele Atlas (www.geocode.com). Pollutant concentrations were obtained from the national archive of ambient air pollution data (the Air Quality System, www.epa.gov/ttn/airs/airsaqs) maintained by the U.S. Environmental Protection Agency. We focused on pollutants relevant to the Fresno environment, including ozone (O_3), nitrogen dioxide (NO_2), carbon monoxide (CO) and particulate matter with a median aerodynamic diameter ≤ 10 μm (PM_{10}).

Pollutant metrics were determined by time-averaging the hourly and daily observed concentrations. All pollutants were measured daily with the exception of PM_{10} which was measured every 6 days. Estimates were mapped to the residences or ZIP code centroids based on inverse distance squared weighting of the monthly average concentrations from the air monitoring stations (up to three) located closest to the residence location. Data from stations within 25 km for CO and 50 km for O_3, NO_2, and PM_{10} were considered in the interpolation.

Skin-prick allergen sensitivity tests were performed with the MultiTest device (donated by Lincoln Laboratory, Decateur, IL) with 14 antigens (Hollister-Stier). Children whose parent reported a severe reaction to a skin test prior to enrollment in FACES were not tested. The histamine control was read 15 minutes after application, all others were read 20 minutes after application. Wheal size was measured as the average of two perpendicular lines, one of which marked the longest dimension of the wheal. A positive test was defined as a wheal ≥ 3 mm larger than the saline control. "Outdoor" antigens included Bermuda grass, olive, ryegrass, Chinese juniper, oak, *Cladosporium*, common privet, mugwort/sagebrush and cedar; "indoor" antigens included house dust mite, cat, dog, *Penicillium*, cockroach. *Alternaria* can be classified as indoor or outdoor, however, for the purposes of these analyses, it was grouped with the indoor allergens. Several months into the study, *Penicillium* was replaced by *Cladosporium* in the skin test panel; therefore, no children were tested with both of these antigens. This antigen panel was chosen to minimize the number of allergens needed to maximize the probability of at least one positive skin test amongst people who would respond to common aeroallergens. Parents were asked to have the children refrain from anti-histamine use for 72 hours prior to the appointment. Although the staff who performed the skin testing were aware of which allergens were applied, they were not aware of the child's exposure to air pollution during any point in life.

To examine the effect of the outdoor pollutants on allergic sensitization, three outcomes were defined. First, sensitization to any allergen was defined as a positive skin test to at least one antigen or a severe reaction to a previous test. Next, sensitization to at least one indoor and at least one outdoor allergens were examined separately. The two additional outcomes were considered, because, despite the fact that elements of the ambient environment (e.g., fine diesel particles) permeate indoors, the exposure to pollutants and allergens in the ambient environment is different from exposure to pollutants and allergens in an indoor environment. Children who had a previous severe reaction were not included in the indoor only and outdoor only analyses, as it was not possible to determine which type of antigen caused the severe reaction.

To define the exposure periods of potential interest, monthly pollutant metrics were averaged separately for the entire prenatal period and each trimester. Trimester dates were determined by the date of birth and the mothers' reports of how many weeks early or late the child was born. Observations with less than 10% of the pregnancy data available were not included. To determine whether any prenatal metrics might just be surrogates for early-life exposure, additional analyses were performed based on exposures from the first year of life and the first 2 years of life. Exposure metrics were limited to these early years of life, because the probability that a child "becomes" sensitized increases as the child ages. Therefore, consideration of exposures during later ages may violate the proper time-ordering required to estimate the influence of these exposures on sensitization status measured at age 6–11 years.

FACES-LiTE was approved by the Committee for the Protection of Human Subjects of the University of California, Berkeley.

Statistical Methods

As noted before, FACES was designed to examine the influence of indoor and outdoor air pollutants on the respiratory health of children with asthma. The effect of outdoor pollutants on allergic sensitization, although a highly relevant research question, was not included in the primary hypotheses when the study was designed. However, our design offered the opportunity to explore this question. In light of this, and due to the relatively small sample size, this evaluation should be considered exploratory in nature, and; therefore many time periods and pollutants were examined to identify possible effects worthy of future study.

Chi-square tests were used to compare the proportions of children who were sensitized across a series of possible confounders. A multi-step modeling strategy was implemented. Because ambient pollutant concentrations decreased over time in our cohort, and the prevalence of sensitization increases with age; the first modeling step included single-pollutant models, adjusted for year-of-birth (YOB). Additional adjustments were considered for YOB-adjusted models with a pollutant term that had a $p < 0.20$. A series of possible confounders and modifiers (listed in Table 2), were considered. These candidate variables were selected based on *a priori* subject-matter knowledge, time-ordering

TABLE 1.—Percent of children positive to each allergen.

% positive to allergen at age 6–11	
Overall (n = 162 skin tested children)	60
Indoor	49
Penicillium notatum (n = 51)	31
Alternaria tenuis	30
Mite (*Dermataphagoides pteronyssinus)*	22
Cat (AP Pelt, Standardized)	17
Cockroach mix (*P. americana and B. germanica)*	11
Dog hair and dander (mixed breeds)	3
Outdoor	44
Bermuda Grass *(Cynodon dactylon)*	29
Olive (*Olea europaea)*	29
Ryegrass, Perenial *(Lolium perenne)*	28
Cladosporium (n = 137)	18
Mugwort, Sagebrush (*Artemisia vulgaris heterophylla)*	18
Common Privet	12
Oak (Red, Virginia Live and White Oak)	11
Cedar (*Juniperus ashei)*	6
Chinese Juniper	2
A child was tested for either penicillium or cladosporium, depending on he/she was entered in the cohort.	

and bivariate associations with the outcomes of interest. Co-pollutants, over the same time period and other intervals, were considered.

Model selection was performed with the Deletion/Substitution/Addition (DSA) routine (http://www.stat.berkeley.edu/~laan/Software/) (13). In the DSA model selection process, the space of candidate predictors was parameterized with four variables that constrain each model considered: the maximum number of terms, the maximum polynomial order, the maximum order of interactions and the maximum sum of powers in each interaction term. For these analyses, the pollutant and YOB terms were included into all models. The models were then restricted to a maximum size of six terms (e.g., YOB and the pollutant metric plus up to four more variables), maximum order of interaction of two and maximum sum of powers of three. The model selection procedure relies on cross-validation with multiple random splits of the data. For a given DSA run, the data splits correspond to the aggregation of k independent 5-fold splits of the data. Stable results were obtained with k = 5. Additional details about the DSA are provided in the on-line repository.

RESULTS

As expected, the prevalence of allergen sensitization was higher than would be found in non-asthmatic populations. Overall, 60% of children were sensitized to at least one allergen and fewer were sensitized to at least indoor allergen (49%) or at least one outdoor allergen (44%) (Table 1). Additional characteristics of the FACES-LITE cohort are displayed in Table 2. Only season-of-birth and mother's age at child's birth were significantly associated with allergen sensitization (Table 2). Children born in the winter were less likely to be sensitized, as were children born to mothers who were over 35 years old at the time of birth.

Trimester-specific metrics are more variable than the entire prenatal or year-long metrics (Figure 1). Correlations between the pollutant levels across different time periods are presented in Table 3. Only the pollutant metrics that are used for regulatory purposes are presented. In general, the strongest correlations are seen between NO_2 and CO, which are both negatively correlated with O_3. Relative to the 1st and 3rd trimester metrics, the 2nd trimester metrics are more highly correlated with the entire pregnancy metrics.

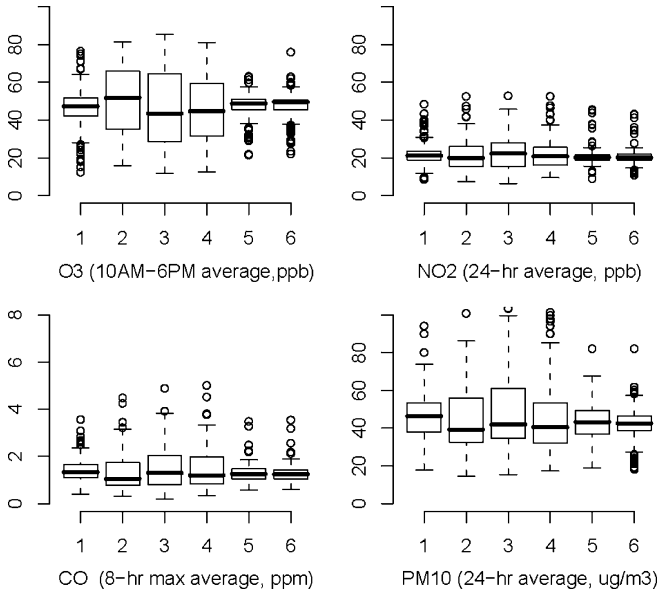

FIGURE 1.—1 = Entire pregnancy, 2 = 1st trimester, 3 = 2nd trimester, 4 = 3rd trimester, 5 = Age 0–1 years, 5 = Age 0–2 years.

When each pollutant metric was regressed on each outcome and adjusted for YOB, a number of significant associations were seen. Sensitization to at least one allergen was associated, in general, with higher levels of CO and PM_{10} during the entire pregnancy and 2nd trimester and higher PM_{10} during the first two years of life. Sensitization to at least *one indoor* allergen was associated with higher exposures to CO and PM_{10} in during the entire pregnancy and during the 2nd trimester. Sensitization to at least one *outdoor allergen* was associated with higher exposure to CO during the entire pregnancy and 2nd trimester. Lower exposure to ozone during the entire pregnancy or 2nd trimester was associated with an increases risk of all three outcomes, perhaps due to the fact that O_3 is negatively correlated with NO_2 and CO.

The DSA model selection algorithm was implemented to adjust for additional possible confounders, effect modifiers and co-pollutants. Several significant associations remained and are presented in Table 4. Higher exposure to CO during the entire prenatal period was associated with an increase sensitization to outdoor allergens. The odds ratio was 1.55 (95% CI: 1.01, 2.37) per interquartile ranges (IQR) increase of 8-hour daily maximum CO during pregnancy. Similar effects estimates were seen for 2nd trimester exposure to CO, but these were less precisely estimated (OR = 1.45, 95% CI: 0.90, 2.35). No significant associations remained for any pollutants and sensitization to any allergen or to an indoor allergen, usually due to the entry of "MALE" into the model. Due to empirical confounding, males were more likely to be sensitized and, due to empirical confounding, also had higher prenatal exposure to CO.

DISCUSSION

Unlike several other published reports (6, 14), sensitization to indoor allergens in our cohort of young children with

TABLE 2.—Characteristics of the FACES-LiTE population according to allergen sensitization.

% of population (based on n = 170)		% sensitized to at least one allergen(based on = 170)	% sensitized to at least one indoor allergen (based on = 162)	% sensitized to at least one outdoor allergen (based on = 162)
Overall Demographics	—	60%	49%	44%
African-American	15	72	54	58
Hispanic	40	64	54	48
White	44	52	43	36*
Sex (male)	57	75*	64*	57*
Family income < 30,000/year	41	60	48	44
Current Exposures				
Smoker in home	16	68	58	46
Currently lives within 4 blocks of major roadway heavy traffic	79	63	51	47
Family History				
Mother had asthma	37	62	49	43
Mother had rhinitis	43	60	48	46
Father had asthma	30	67	50	45
Father had rhinitis	36	58	50	40
Birth/Prenatal Characteristics				
Low birth weight or premature	13	57	55	40
First born child	45	66	56	48
Mother smoked when pregnant	8	54	31	38
Ever breastfed	73	60	45	44
Born in CA	98	60	47	43
Born in Fresno	81	60	48	42
Pollutant season of birth				
Winter (Oct-Jan)	30	49*	38	30*
Spring (Feb-May)	41	63	52	52*
Summer (June-Sept)	28	67	59*	47
Mother's age at birth				
<18 years	5	88**	88	63
19–34 years	80	62**	48**	45**
>=35 years	15	36**	30**	22**
Child's health history				
Rhinitis	31	58	48	35
Eczema	12	60	59	53
Asthma diagnosed at <= 2 yrs old	40	54	41	37

*$p < 0.05$ for chi-square (e.g., one season vs. all other seasons), **$p < 0.05$, test for trend.

asthma was not substantially higher than sensitization to outdoor allergens. This population of asthmatic children has a higher prevalence of sensitization to common aeroallergens than the general population (15). Similarly, the frequency of roach sensitization was lower than in other urban asthmatic populations (16), perhaps because Fresno has a dry climate (<10" rain per year, climate-zone.com) and less large-scale public housing than other urban areas, which provides a less suitable habitat for roaches.

In multivariable analyses, the outdoor pollutants were not associated with sensitization to indoor allergens or the combined outcome of sensitization to any indoor or outdoor allergens. Outdoor pollutants may play a lesser role in sensitization to indoor allergens for several reasons. Outdoor pollutants examined are likely to penetrate indoors in varying degrees due to housing characteristics and activities such as cooking and fireplace use also influence their concentrations within the home. Information was not available to evaluate whether, for example, parents (particularly those with allergies) may have attempted to reduce exposure to indoor allergens, thereby influencing the pathway between exposure and sensitization.

In contrast, in multivariable analyses, the influence of prenatal exposure to CO on the sensitization to outdoor allergens remained and was more pronounced for 2nd trimester exposures, relative to the 1st and 3rd trimesters exposures or those from the first year or two of life. Based on a rhesus monkey model of the development of higher primary immune function in fetal life, the second trimester is an important period of development of lymphoid tissues and immune regulatory and effector function (17). In humans, the 2nd trimester also is a time during which the cellular machinery involved in IgE-mediated response is reaching a high level of development, although there are very few data on the ability of the fetus to react to antigen exposure during this trimester and thereby primed for post-natal reactivity (18).

That the fetus is capable of immunological response to aeroallergens at some point during development has been demonstrated based on studies with cord blood mononuclear cells (10). Thus, while our observations could be due to chance or the correlation with total exposure during the pregnancy, especially in light of the lack of association with 3rd trimester exposure, our findings are consistent with alteration of developmental steps that could lead to skewing of immune responses during later fetal development and in the early postnatal period (19), either by enhancing the response of the more developed 3rd trimester immune competence or by further delay the perinatal loss of the Th2-skewed state that characterizes pregnancy (18).

The evidence that air pollution causes asthma is limited (20). However, our findings suggest, that among asthmatics, air pollution may contribute to the risk of immunological

TABLE 3.—Correlations between regulatory pollutant metrics for prenatal and early-life intervals.

	CO 8-hour max	NO_2 24-hour average	O_3 8-hour max	PM_{10} 24-hour average	CO 8-hour max	NO_2 24-hour average	O_3 8-hour max	PM_{10} 24-hour average
		Entire Prenatal				Trimester 1		
Entire Prenatal								
CO 8-hour max	1.0	0.74	−0.40	0.32	.42	.49	0.10	0.22
NO_2 24-hour avg	—	1	−0.30	0.35	.29	.69	0.12	0.22
O_3 8-hour max	—	—	—	0.31	−0.25	−0.36	0.33	0.03 (NS)
PM_{10} 24-hour avg	—	—	—	—	0.05 (NS)	0.20	0.38	0.56
		Trimester 2				Trimester 3		
Entire Prenatal								
CO 8-hour max	0.75	0.68	−0.26	0.32	0.48	0.37	−0.26	0.01 (NS)
NO_2 24-hour avg	0.54	0.82	−0.25	−.30	0.42	0.60	−0.27	0.10 (NS)
O_3 8-hour max	−0.33	−0.29	0.67	0.14	−0.04 (NS)	0.12 (NS)	0.32	0.44
PM_{10} 24-hour avg	0.31	0.37	0.09 (NS)	0.75	0.17	0.15	−0.02 (NS)	0.58
		Age 0–1 years				Age 0–2 years		
Entire Prenatal								
CO 8-hour max	0.65	0.51	−0.19	0.14	0.68	0.53	−0.20	0.17
NO2 24-hour avg	0.52	0.74	−0.21	0.25	0.55	0.72	−0.23	0.24
O3 8-hour max	−0.11 (NS)	−0.20	0.49	0.24	−0.17	−0.21	0.51	0.28
PM10 24-hour avg	0.15	0.09 (NS)	0.28	0.57	0.26	0.15	0.25	0.69

All correlations have a p-value < 0.05 unless noted with NS—not significant.

TABLE 4.—Single-pollutant models for "sensitized to at least one outdoor allergen."

			Adjusted for year of birth and sex		
	Interquartile range (IQR)	Crude Coefficient	Coefficient	Standard Error	Odds ratio (95% CI) Per IQR increase
Entire pregnancy					
CO, 24-hr average	0.28	1.70	1.33	0.68	1.45 (1.02, 2.07)
CO, daily max	0.79	0.68	0.54	0.27	1.53 (1.01, 2.33)
CO, 8-hour max	0.52	1.07	0.84	0.42	1.55 (1.01, 2.37)
2nd trimester					
CO, 24-hr average	0.73	0.71	0.57	0.34	1.52 (0.93, 2.47)
CO, daily max	1.93	0.26	0.21	0.13	1.50 (0.92, 2.45)
CO, 8-hour max	1.17	0.41	0.32	0.21	1.45 (0.90, 2.35)

response to outdoor allergens. Allergic sensitization defined by skin test results often is associated with more severe asthma (21, 22). Therefore, even if air pollution does not 'cause' asthma, it may contribute to asthma exacerbations through sensitization to some allergens. Human exposure studies have shown that diesel exhaust particles can enhance IgE-mediated responses to allergens (9), an observation that is relevant to our population, since diesel exhaust is an important source of air pollution in the study community (23, 24).

Although CO is most likely a surrogate for mobile sources emissions in our study area, CO itself may interact with other pollutants such as O_3 to enhance responses to environmental antigens (25). Moreover, while NO_2 has been shown to capable of enhancement of allergic responses to aeroallergens (26), it is more likely that the observed associations with CO are related to their association with exposures to other elements of mobile source pollution. In particular, diesel exhaust particles are be show to alter IgE responses aeroallergens *in vitro* and *in vivo*[9] and diesel engines are a major source of oxides of nitrogen (27). Fresno is bisected (NW to SE) by Interstate 99 that has a very high daily volume of light and heavy duty diesel trucks (28). In addition, gasoline engines contribute large amounts of polycylic aeromatic hydrocarbons that also enhance IgE-specific immune response. In this latter regard, Fresno is bisected (N to S) by Interstate 41 that has a very high volume daily volume of cars (28). Finally, *in vitro* studies have indicated that NO_2 enhances the release of the antigenic proteins contained in pollen grains (26). Since ambient CO in Fresno comes from motor vehicles as does most of the NO_2 and the two pollutant concentrations were moderately correlated over the entire prenatal period, these latter NO_2 effects on pollen release could be contributing to the associations between CO and sensitization.

We are not aware of any studies of the effect of air pollution on allergen sensitization that were restricted to children with asthma, or any that considered prenatal exposures, and therefore, it is difficult to compare our findings with other published reports. Corbo, *et al*. reported that air pollution did not increase risk of atopy but exacerbated symptoms in already sensitized people (29). This study was, however, a cross-sectional comparison of children from three areas with different pollutant profiles. Brauer *et al.* analyzed exposures of non-asthmatics during the first four years of life and found positive associations between air pollution and the following endpoints: wheeze, asthma, ear/nose throat infections, serious colds, and sensitization to food allergens, but not total IgE (30). Sensitization defined by skin-test results was not examined in this cohort, nor was prenatal exposure. Hirsch *et al*. found an association with the prevalence of cough and bronchitis, but not with atopic conditions (including skin test results) in children, but did not consider prenatal exposures (4).

In Fresno, O_3 is negatively correlated with NO_2 and CO, which are positively correlated with each other. NO_2 and CO are markers for pollutants generated by urban combustion sources, primarily mobile sources in Fresno. Most of the residences are located in the urban and suburban areas that have elevated NO_2 concentrations and moderate O_3 concentrations. Few study participants lived in the downwind areas (e.g., near Parlier, CA) that have high O_3 and low NO_2 and CO concentrations. The absence of rural downwind exposures in our study population probably explains the lack of O_3 association with the outcome. The NO_2 and CO associations are stronger than the O_3 association in this urban setting, where substantial scavenging of O_3 by fresh NO emissions occurs.

Our findings are important for several reasons. Sources of outdoor allergens are more seasonal than indoor allergens, such as those derived from pets that live in a home yearround. While exposure to indoor allergens may be more frequent, the sources and levels of indoor allergens are more easily controlled. In the short-term, exposure to outdoor allergens can be controlled by remaining indoors under closed ventilation conditions that exclude these allergens. However, unless one moves to a community with lower outdoor allergen levels or installs expensive home filtration equipment, there is little that can reduce exposure to outdoor allergens over a prolonged period such as the prenatal months. Consequently, sensitivity to these allergens may result in more symptoms and asthma exacerbations then sensitization to indoor allergens. These results demonstrate that sensitization to indoor and outdoor allergens should be examined as separate outcomes.

Our data demonstrate that effects of prenatal exposures can be seen among school-aged children. As reported in the second-hand tobacco smoke exposure literature, it is difficult to disentangle the effect of prenatal and postnatal exposures. Our models suggest that prenatal exposures increased sensitization to outdoor allergens later in life—more than exposures restricted to the first 2 years of life or lifetime metrics (data not shown), observations that are consistent with those reported for second-hand exposure (31). Although the pollutant metrics across time periods are correlated, the strongest associations with the outcomes

were seen for prenatal exposures. When lifetime metrics were considered alone, or in combination with the prenatal metrics, the lifetime measures were not at all associated with any of the outcomes (data not shown). This suggests the timing of the exposure may be more important than the overall dose and prenatal exposures are not just markers for lifetime or current exposures. It should be noted that the greater variability of trimester exposures may increase the power to detect the associations that exist.

There are limitations to this study and to our analytic approach. Exposure assignments are based solely on fixed-site monitoring data; therefore, misclassification is a concern. However, the misclassification is likely to be non-differential, that is, not related to the outcome of interest and, therefore, is likely to bias to toward the null. In addition, the use of personal rather than fixed-site monitors to characterize trimester-specific exposures among asthmatic children would be extremely expensive or nearly impossible, since only a small percentage of pregnancies would lead to the birth of a child who would be diagnosed with asthma by the age of 6–11 years.

Although it is possible that the observed significant negative health findings are confounded, this was addressed through the use of a modeling strategy that considered a wide range of confounders and modifiers. Even when all co-pollutants and demographic characteristics were considered for entry into the model, only year of birth and male were ever selected, which suggests little confounding existed.

The associations are based on observational data and cannot necessarily be interpreted as causal effects. Because the pollutants were forced into the model, the metrics could be serving as a marker for another correlated pollutant that was not considered or did not enter the model because the force variable was already contributing to the prediction of that outcome. However, both NO_2 and CO are traffic-generated pollutants, and as such, interventions to reduce their effect, or the effect of an unmeasured correlated pollutant, are likely to be similar.

Recall of exact residence during pregnancy could be problematic. However, most of the biological mothers completed the interview and are likely to remember at least the city of residence during pregnancy. Given that exposures are based on central-monitors, city information is likely to be adequate. Inability to recall a prenatal address is not likely to be related to sensitization at age 6–11 years, and therefore not account for our findings.

Daily allergen data during this time period are not available. However, a comparison of daily data from 2001–2003 in Fresno found, for example, 24-hour average CO was uncorrelated with total pollens ($r = -0.09$), total agricultural fungi ($r = -0.18$), and *Alternaria* ($r = 0.03$). Stronger correlations were seen with total fungal spores ($r = 0.30$), *Cladosporium* ($r = 0.34$) and *Aspergillius* ($r = 0.26$). Perhaps those allergens, alone or in combination with these traffic-related pollutants may contribute to sensitization. However, the associations were seen for 2nd trimester, and the months included in that trimester vary across children due to differences in birth dates. So, while annual or season-specific allergen and pollutant metrics could be collinear, it is difficult to imagine a scenario in which the *trimester* values of a given pollutant would co-vary with an allergen.

These analyses included a large series of comparisons across time periods and pollutants. Therefore, the occurrence of "significant" associations due to multiple comparisons may be of concern. As noted in the Methods section, however, this should be viewed as an exploratory, hypothesis-generating undertaking. The findings may motivate a more extensive examination of the effect of traffic-related pollutant exposure during pregnancy on allergen sensitization. In particular, the consistency of the findings form the basis of our inference that future research of this question is warranted, despite the imprecision of those estimates. No significant effect modifiers were identified, however, it is likely these exploratory analyses lacked sufficient power to detect possible effect modification by factors such as parental allergen sensitization status, season of birth, housing characteristics such as gas stoves, and gender. Studies among more geographically diverse populations with larger exposure gradients may identify additional effects. Future prospective studies in larger populations are warranted to assess the timing of sensitization and identify possible susceptible subgroups. Studies within non-asthmatic cohorts of children would be useful to determine if their response to prenatal exposures is similar to this cohort with asthma.

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ABBREVIATIONS

CARB	California Air Resources Board
CO	Carbon Monoxide
DSA	Deletion/Substitution/Addition algorithm
FACES	Fresno Asthmatic Children's Environment Study
FACES-LiTE	FACES –Lifetime Exposure
NO_2	Nitrogen Dioxide
O_3	Ozone
PM_{10}	Particulate matter with a median aerodynamic diameter $<10\mu$

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