Air pollution and IgE sensitization in 4 European birth cohorts—the MeDALL project

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Background: Whether long-term exposure air to pollution has effects on allergic sensitization is controversial.
Objective: Our aim was to investigate associations of air pollution exposure at birth and at the time of later biosampling with IgE sensitization against common food and inhalant allergens, or specific allergen molecules, in children aged up to 16 years.
Methods: A total of 6163 children from 4 European birth cohorts participating in the Mechanisms of the Development of ALLergy [MeDALL] consortium were included in this meta-analysis of the following studies: Children, Allergy, Milieu, Stockholm, Epidemiology (BAMSE) (Sweden), Influences of Lifestyle-Related Factors on the Human Immune System and Development of Allergies in Childhood (LISA)/German Infant Study on the Influence of Nutrition Intervention PLUS Environmental and Genetic Influences on Allergy Development (GINIplus) (Germany), and Prevention and Incidence of Asthma and Mite Allergy (PIAMA) (The Netherlands). The following indicators were modeled by land use regression: individual residential outdoor levels of particulate matter with aerodynamic diameters less than 2.5 μm, less than 10 μm, and between 2.5 and 10 μm; $PM_{2.5}$ absorbance (a measurement of the blackness of $PM_{2.5}$ filters); and nitrogen oxides levels. Blood samples drawn at ages 4 to 6 (n = 5989), 8 to 10 (n = 6603), and 15 to 16 (n = 5825) years were analyzed for IgE sensitization to allergen extracts by ImmunoCAP. Additionally, IgE against 132 allergen molecules was measured by using the MedALL microarray chip (n = 1021).
Results: Air pollution was not consistently associated with IgE sensitization to any common allergen extract up to age 16 years. However, allergen-specific analyses suggested increased risks of sensitization to birch (odds ratio [OR] = 1.12 [95% CI = 1.01-1.25] per 10-μg/m^3 increase in NO_2 exposure). In a subpopulation with microarray data, IgE to the major timothy grass allergen *Phleum pratense* 1 (Phl p 1) and the cat allergen *Felis domesticus* 1 (Fel d 1) greater than 3.5 Immuno Solid-phase Allergen Chip standardized units for detection of IgE antibodies were related to $PM_{2.5}$ exposure at birth (OR = 3.33 [95% CI = 1.40-7.94] and OR = 4.98 [95% CI = 1.59-15.60], respectively, per 5-μg/m^3 increase in exposure).
Conclusion: Air pollution exposure does not seem to increase the overall risk of allergic sensitization; however, sensitization to birch as well as grass pollen Phl p 1 and cat Fel d 1 allergen molecules may be related to specific pollutants. (J Allergy Clin Immunol 2020;■■■:■■■-■■■.)

Key words: *Allergy, allergen, air pollution, children, cohort, IgE, sensitization, meta-analysis*

Abbreviations used

BAMSE:	Children, Allergy, Milieu, Stockholm, Epidemiology
ESCAPE:	European Study of Cohorts for Air Pollution Effects
Fel d 1:	*Felis domesticus* 1
GINIplus:	German Infant Study on the Influence of Nutrition Intervention PLUS Environmental and Genetic Influences on Allergy Development
ISAC:	Immuno Solid-phase Allergen Chip
ISU-E:	Immuno Solid-phase Allergen Chip standardized units for detection of IgE antibodies
LISA:	Influences of Lifestyle-Related Factors on the Human Immune System and Development of Allergies in Childhood
LUR:	Land use regression
MeDALL:	Mechanisms of the Development of ALLergy
NO_2:	Nitrogen dioxide
NO_x:	Nitrogen oxides
OR:	Odds ratio
Phl p 1:	*Phleum pratense* 1
PIAMA:	Prevention and Incidence of Asthma and Mite Allergy
$PM_{2.5}$:	Mass concentration of particles less than 2.5 μm in size
PM_{10}:	Mass concentration of particles less than 10 μm in size
$PM_{2.5}$ absorbance:	Measurement of the blackness of $PM_{2.5}$ filters
PM_{coarse}:	Mass concentration of particles between 2.5 and 10 μm in size

Associations between exposure to outdoor air pollution and several adverse health conditions in children and adolescents, including lower lung function and higher respiratory morbidity, have been widely demonstrated[1-4]; however, the evidence for associations of air pollution with the risk of allergic diseases remains conflicting because of the small number of studies available in the literature and their inconsistent results. Experimental studies provide a biologic basis for air pollutants being risk factors for allergic sensitization by demonstrating enhanced IgE production following exposure to particulates.[5-7] To date, there have been only a few prospective cohort studies following children from birth up to school age, with objective assessments of specific sensitization to allergen extracts as well as assessments of exposure to air pollution at an individual level.[8-13] In an earlier multicohort analysis with harmonized exposure and health data from 5 European cohorts (including the 4 cohorts participating in this analysis), we found no apparent association between exposure to air pollution and allergic sensitization in children at either 4 years or 8 years of age.[14] Recent narrative and systematic reviews evaluating the body of evidence on air pollution exposure and allergy outcomes have provided inconclusive results, acknowledging a high degree of heterogeneity between existing studies.[15-17] Importantly, most previous studies have been limited to cross-sectional analyses of prevalence of specific IgE sensitization measured only up to school age. In a longitudinal analysis of 2 German birth cohorts that were followed for 10 years, no consistent evidence that exposure to air pollution increases the risk of aeroallergen sensitization in later childhood was found.[13] To our knowledge, as yet there has been no combined longitudinal analysis of the relationship between air pollution exposure and prevalence of IgE sensitization against common allergens or defined allergen molecules in individuals up to 16 years of age based on prospective birth cohort studies.

Therefore, we conducted the present study within the framework of the European collaborative Mechanisms of the Development of ALLergy (MeDALL)[18] project with the aim of evaluating the association of exposure to air pollution with prevalence of IgE sensitization in children during the first 16 years of life. We implemented a meta-analysis based on already collected and harmonized health data from 4 European birth cohorts and applied uniform exposure assessment methodology developed

TABLE I. Overview of the tested allergen sources in the 4 birth cohorts

Cohort	Test system	Tested allergens
BAMSE	ImmunoCAP System, Thermo Fisher/Phadia AB, Uppsala, Sweden (Phadiatop/fx5)	Inhalant allergen sources: ● Outdoor: birch, timothy grass, mugwort ● Indoor: cat, dog, mold (*Cladosporium herbarum*), house dust mite (Der p) Food allergen sources: cow's milk, egg white, soy bean, peanut, cod fish and wheat
PIAMA	Radioallergosorbent test–like method used at the Sanquin Laboratories (Amsterdam, The Netherlands)	Inhalant allergen sources: ● Outdoor: birch, dactylis glomerata ● Indoor: cat, dog, *Alternaria alternata,* house dust mite (Der p) Food allergen sources: egg, milk
LISA/GINI South, LISA/GINI North	CAP-RAST FEIA (Pharmacia Diagnostics, Freiburg, Germany): SX1/FX1	Inhalant allergen sources: ● Outdoor: birch, timothy grass, mugwort ● Indoor: cat, dog, mold (*Cladosporium herbarum*), house dust mite (Der p) Food allergen sources: cow's milk, egg white, soy bean, peanut, cod fish, rye, and wheat

Der p, *Dermatophagoides pteronyssinus*.

within the European Study of Cohorts for Air Pollution Effects (ESCAPE) project (http://www.escapeproject.eu).[19] Furthermore, the availability of MeDALL microarray data on IgE against 132 allergen molecules[20] in 2 of the cohorts (Children, Allergy, Milieu, Stockholm, Epidemiology [BAMSE] and German Infant Study on the Influence of Nutrition Intervention PLUS Environmental and Genetic Influences on Allergy Development [GINIplus]) provided us for the first time with a unique opportunity to investigate associations of ambient air pollution with sensitization to defined inhalant outdoor and indoor allergen molecules, as well as to food and venom allergen molecules. Finally, we investigated the importance of timing of long-term exposure to air pollution by utilizing information on exposures early in life and at the time of later biosampling.

METHODS

The Methods section in this article's Online Repository (available at jacionline.org) provides additional details on the study populations, assessment of exposure to air pollution, IgE microarray measurement, and statistical analyses used in this study.

Study populations

The current study included data from the following 4 European birth cohorts participating in the MeDALL project: BAMSE (Sweden), Prevention and Incidence of Asthma and Mite Allergy (PIAMA [The Netherlands]), Influences of Lifestyle-Related Factors on the Human Immune System and Development of Allergies in Childhood (LISA [Germany]), and GINIplus (Germany). Participants were recruited from 1994 to 1999. Detailed descriptions of study design, enrollment, and procedures for data collection in each cohort have been published elsewhere.[21-23]

Air pollution exposure assessment

Annual mean concentrations of ambient particulate matter with an aerodynamic diameter less than 2.5 μm ($PM_{2.5}$) or 10 μm (PM_{10}), coarse particulate matter ($PM_{2.5\text{-}10}$), $PM_{2.5}$ absorbance (an indicator for black carbon particulate matter content), nitrogen dioxide (NO_2), and nitrogen oxides (NO_x) at the residential addresses of the study participants were estimated through land use regression (LUR) models developed for each study area within the framework of the ESCAPE project; they have been extensively described elsewhere,[24,25] as well as in the Online Repository.

Measurement of IgE sensitization

At the age of 4 years (6 years for the LISA/GINI cohorts), 8 years (10 years for the LISA/GINI cohorts), and 16 years (15 years for the LISA/GINI cohorts) children were invited for clinical examinations including biosampling. Blood samples were drawn for the analysis of IgE sensitization by measuring specific serum IgE levels against panels of common inhalant and food allergen sources (natural allergen extracts) by using the ImmunoCAP System (Thermo Fisher/Phadia AB, Uppsala, Sweden) or equivalent test systems (ie, a radioallergosorbent test–like method used at the Sanquin Laboratories [Amsterdam, The Netherlands] and the CAP-RAST FEIA [Pharmacia Diagnostics, Freiburg, Germany]). The range of tested inhalant allergen sources was comparable across the studies and included cat, dog, mold, house dust mite, birch pollen, and grass pollen for all cohorts, as well as mugwort for all cohorts except PIAMA (Table I). The panel of food allergen sources includes cow's milk and egg for all cohorts and soy bean, peanut, cod fish, and wheat, in all cohorts except PIAMA (milk and egg only). Details regarding the set of specific allergens tested in each cohort are given in Table I. Sensitization was defined as an IgE antibody level of at least 0.35 kU_A/L to any of the tested allergen extracts.

In addition, IgE sensitization to 132 allergen molecules corresponding to 51 allergen sources was analyzed in BAMSE at the age of 16 years (n = 743), as well as in GINIplus at the age of 15 years (n = 278); the analysis was performed with an allergen chip based on Immuno Solid-phase Allergen Chip (ISAC [Thermo Fisher]) standardized units for detection of IgE antibodies technology (ISU-E), which was developed within the MeDALL project.[26] Serum aliquots of 30 μL were incubated on the microarray for 2 hours at room temperature, and the slides were washed and then incubated with fluorescence-labeled anti-IgE antibodies (Thermo Fisher) for 30 minutes. The chips were then washed, dried, and analyzed with a Laser Scan Confocal microarray reader (LuxScan 10K/A [Capital-Bio, Beijing, China]). A complete list of included molecules and the prevalence of IgE sensitization against those in the 2 cohorts are presented in Table E1 (available in the Online Repository at www.jacionline.org). The cutoff for IgE detection was set at 0.3 ISU-E.

Statistical analyses

Associations of exposure to outdoor air pollution with prevalence of sensitization until 16 years of age were assessed in each cohort separately by using generalized estimating equation models with an unstructured correlation structure to account for correlations between repeated observations within the same subject. Separate analyses were conducted with exposure at the birth address and at the address at which the participant lived at the time of biosampling. The models incorporated interaction terms between time

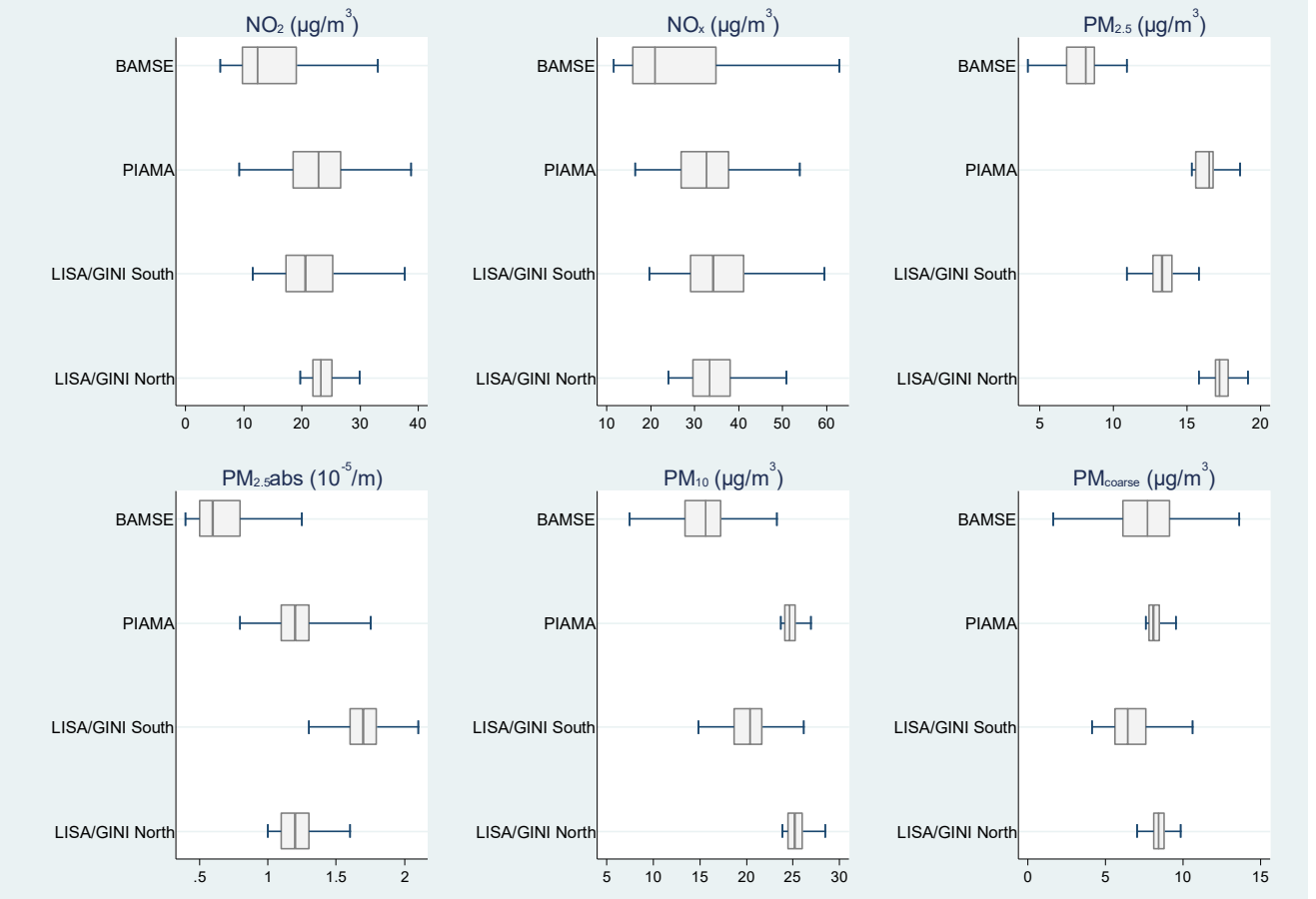

FIG 1. Air pollution exposure at birth address in 4 European birth cohorts. Each box contains the middle 50% of the data, with the right edge (*hinge*) of the box indicating the 75th percentile and the left edge indicating the 25th percentile (interquartile range). The line in the box represents the median. The ends of the horizontal lines ("whiskers") indicate 1.5 × IQR.

indicator variable (age) and exposure to evaluate age-specific effects of exposure.

Adjusted cohort-specific odds ratios (ORs) and 95% CIs were then meta-analyzed by using a random effects model,[27] taking into account possible nonrandom variability within and between cohorts. Statistical heterogeneity among studies was evaluated by using I^2 statistics.[28] A P value less than .05 was considered significant. The results of microarray-wide analyses were adjusted for multiple testing by using Bonferroni correction applied to 132 tests.[29] All analyses were performed with STATA software, release 13.1 (StataCorp, College Station, Tex).

RESULTS

The population of the present study comprises individuals with available data on repeated IgE measurements throughout childhood and adolescence and air pollution exposure at birth (n = 6163) or at the time of biosampling (n = 5771). A short description of selected characteristics related to the enrolment and follow-ups of the included cohorts is provided in Table E2 (available in the Online Repository at www.jacionline.org). The distribution of potential risk factors at the ages of 4 to 6, 8 to 10, and 15 to 16 years in each cohort is presented in Tables E3 to E5, respectively (available in the Online Repository at at www.jacionline.org). Notably, in the subset of children with IgE measurements in the BAMSE cohort, the proportion of children with allergic heredity was lower than in the other cohorts. This is partly explained by differences in study designs between the cohorts. Fig 1 presents the distribution of air pollution exposure concentrations at the participants' birth addresses. For most pollutants, the levels were lower for the Stockholm County area, which is where the BAMSE cohort is based. Median air pollution levels ranged from 12.4 μg/m^3 (BAMSE) to 23.2 μg/m^3 (LISA/GINI North) for NO_2 and from 8.1 μg/m^3 (BAMSE) to 17.2 μg/m^3 (LISA/GINI North) for $PM_{2.5}$, whereas the concentrations of PM_{coarse} were largely comparable between the cohorts. The levels of air pollution at home addresses at the time of respective biosampling are summarized in Table E6 (available in the Online Repository at www.jacionline.org). There were no major changes in estimated air pollution levels at the addresses at different ages. Although more than half of the study subjects moved at least once during follow-up, exposure levels at the birth address and at the follow-up addresses were moderately to highly correlated (see Fig E1 in the Online Repository at www.jacionline.org). The strongest correlations were observed for the LISA/GINI North cohort (r = 0.59-0.77 for the 15-year addresses) and lowest for the BAMSE cohort (r = 0.35-0.45 for the 16-year addresses).

In the 4 cohorts, the prevalence of sensitization to a combination of common inhalant and food allergen extracts

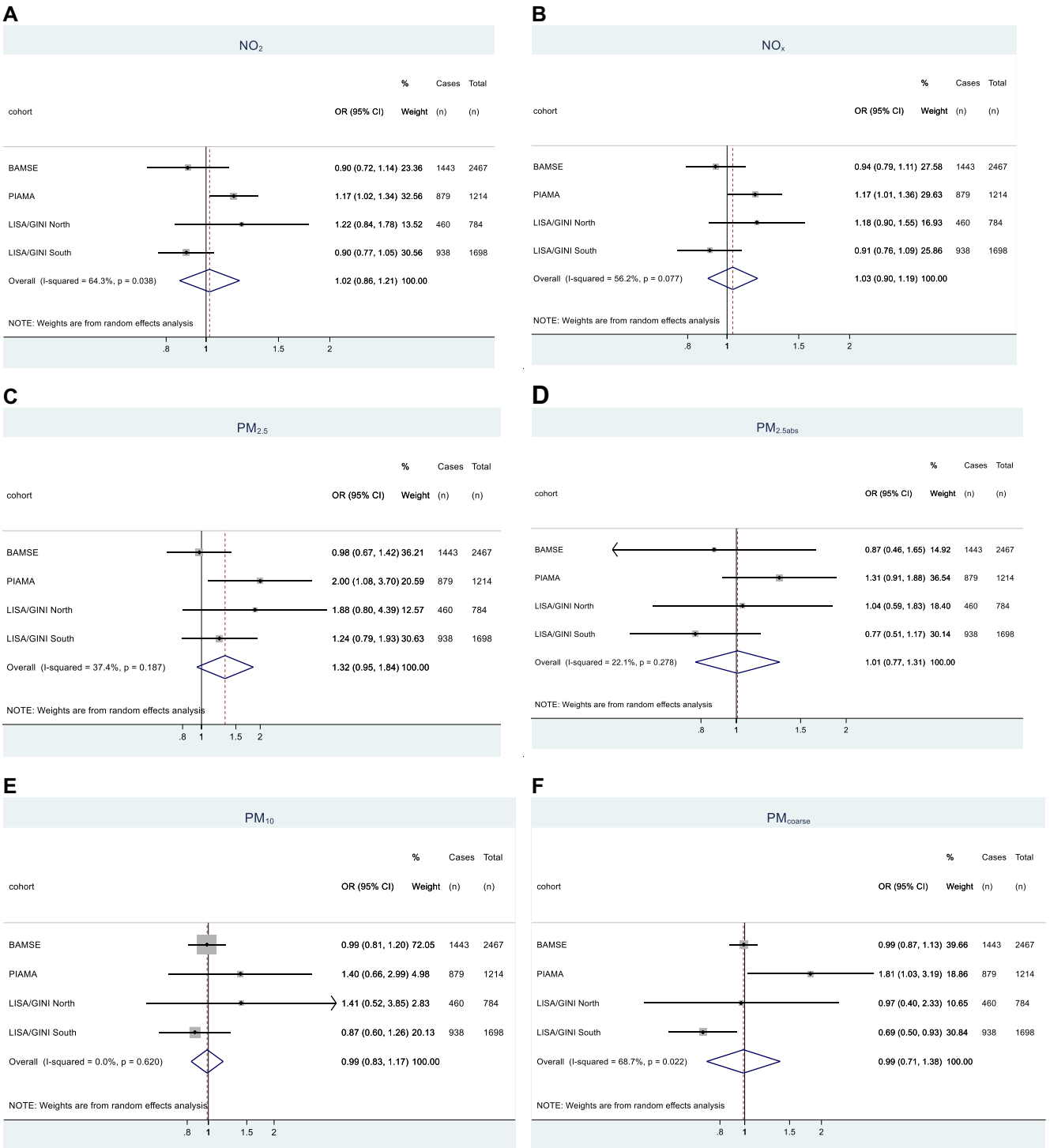

FIG 2. Cohort-specific and combined associations of air pollution exposure at birth with IgE sensitization to any common inhalant and/or food allergen extract up to 16 years of age in 4 European birth cohorts (n = 6163): NO_2 (**A**), NO_x (**B**), $PM_{2.5}$ (**C**), $PM_{2.5}$ absorbance (**D**), PM_{10} (**E**), and PM_{coarse} (**F**). Adjusted for sex, maternal smoking during pregnancy, anyone smoking at the child's home, breast-feeding, atopic parents, parental education, mold at home, furred pets at home, older siblings, gas cooking, study arm (GINIplus cohort), and region (for the BAMSE cohort only). Combined ORs and 95% CIs have been derived by random effects methods. Effects are presented for increments of 10 $\mu g/m^3$ (NO_2, PM_{10}), $1\times10^{-5}/m$ ($PM_{2.5}$ absorbance), 5 $\mu g/m^3$ ($PM_{2.5}$, PM_{coarse}), and 20 $\mu g/m^3$ (NO_x).

TABLE II. Meta-analyses of the associations between air pollution exposure at birth or at the time of biosampling and IgE sensitization to selected pollen allergen extracts up to age 16 years in 4 European birth cohorts

	Birch pollen extract			Timothy grass pollen extract		
Air polution indicator	**OR**	**95% CI**		**OR**	**95% CI**	
At birth						
Cases (no./total no.)		1494 of 6163			1965 of 6163	
NO_2	0.99	0.83	1.18	0.99	0.86	1.15
NO_x	0.98	0.84	1.15	1.00	0.87	1.15
$PM_{2.5}$	1.25	0.79	1.97	1.20	0.88	1.63
$PM_{2.5}$ absorbance	0.97	0.66	1.42	0.94	0.71	1.25
PM_{10}	0.97	0.77	1.22	1.03	0.83	1.27
PM coarse	0.89	0.62	1.30	0.94	0.73	1.20
At the time of biosampling						
Cases (no./total no.)		1409 of 5771			1842 of 5771	
NO_2	**1.12**	**1.01**	**1.25**	0.99	0.88	1.10
NO_x	1.09	0.99	1.20	0.99	0.89	1.11
$PM_{2.5}$	1.21	0.98	1.49	1.25	0.87	1.82
$PM_{2.5}$ absorbance	1.07	0.83	1.38	1.03	0.78	1.36
PM_{10}	**1.24**	**1.03**	**1.50**	1.11	0.85	1.44
PM coarse	**1.23**	**1.07**	**1.40**	1.05	0.94	1.18

Effects are presented for increments of 10 $\mu g/m^3$ (NO_2, PM_{10}), 1×10^{-5}/m ($PM_{2.5}$ absorbance), 5 $\mu g/m^3$ ($PM_{2.5}$, PM_{coarse}), and 20 $\mu g/m^3$ (NO_x). Adjusted for sex, maternal smoking during pregnancy, anyone smoking at the child's home, breast-feeding, atopic parents, parental education, mold at home, furred pet at home, older siblings, gas cooking, study arm (for GINIplus cohort), and region (for the BAMSE cohort only). Boldface indicates statistical significance.

ranged from 24.1% to 40.4% at the age of 4 to 6 years (the lowest rates were in the BAMSE cohort and the highest in the PIAMA cohort), from 34.8% to 47.9% at the age of 8 to 10 years (in the BAMSE and LISA/GINI South cohorts, respectively), and from 41.8% to 51.2% at the age of 15 to 16 years (in the LISA/GINI North and LISA/GINI South cohorts, respectively), as shown in Table E7 (available in the Online Repository at www.jacionline.org).

Cohort-specific and combined overall associations of air pollution exposure at birth and at the time of biosampling and IgE sensitization to mixes of common inhalant and/or food allergen extracts up to 15 to 16 years are shown in Fig 2 and Fig E2 (available in the Online Repository at www.jacionline.org), respectively. The heterogeneity between cohort-specific effect estimates was generally moderate (I^2 = 0-72%). In general, no consistent evidence was found for either exposure at birth or at the time of biosampling to be associated with sensitization to any common allergen up to 15 or 16 year of age. In the cohort-specific analyses, we observed statistically significant positive associations with several air pollutants in the PIAMA cohort, but not in the other included cohorts. The combined adjusted meta-analysis ORs for air pollution exposure at birth ranged from an OR of 0.99 (95% CI = 0.83-1.17) for a 10-$\mu g/m^3$ increase in PM_{10} exposure to an OR of 1.32 (95% CI = 0.95-1.84) for a 5-$\mu g/m^3$ increase in $PM_{2.5}$ exposure. Similar results were obtained in relation to air pollution exposure at the time of biosampling (see Fig E2). The results of the fully adjusted analyses were largely comparable to those based on the basic model (see Table E8 in the Online Repository at www.jacionline.org). Further, increasing cutoffs for IgE positivity to any of the tested inhalant and/or food allergen extracts (ie, ≥0.70 kU_A/L and ≥3.5 kU_A/L) had no major impact on the overall results (see Table E9 in the Online Repository at www.jacionline.org). On the basis of analyses with exposure-age interaction terms, we did not find any indication of differences in age-specific associations with either exposure at birth or exposure at the time of biosampling (see Table E10 in the Online Repository at www.jacionline.org). When we distinguished between sensitization against mixes of food and inhalant allergen extracts, there was a general trend of higher risk estimates for food allergens in relation to all studied pollutants, although the corresponding CIs largely overlapped (see Tables E11 and E12 in the Online Repository at www.jacionline.org). However, by looking separately at associations with sensitization against single-allergen extracts we observed statistically significantly higher odds of sensitization against birch pollen extract for exposure at the time of biosampling to several markers of air pollution (adjusted meta-analysis OR = 1.12 [95% CI = 1.01-1.25] for a 10-$\mu g/m^3$ increase in NO_2 exposure; adjusted meta-analysis OR = 1.24 [95% CI = 1.03-1.50] for a 10-$\mu g/m^3$ increase in PM_{10} exposure; and adjusted meta-analysis OR = 1.23 [95% CI = 1.07-1.40] for a 5-$\mu g/m^3$ increase in PM_{coarse} exposure [Table II and see Table E13 in the Online Repository at jacionline.org]). No consistent association was found with sensitization against timothy grass pollen extract (see Table E14 in the Online Repository at www.jacionline.org).

Further, we evaluated the association of air pollution exposure with IgE sensitization to microarrayed allergen molecules at the age of 15 to 16 years as determined with the MeDALL chip. In the initial discovery microarray-wide association meta-analysis based on the BAMSE and GINIplus cohorts, no allergen molecule appeared to be significantly associated with air pollution exposure after multiple testing corrections (data not shown). In the candidate analysis, which was limited to 4 "risk molecules" previously linked to the risk of respiratory allergy,[20] we observed positive associations of sensitization against these molecules with $PM_{2.5}$ exposure at birth address that did not reach the level of statistical significance (see Table E15 in the Online Repositoryat www.jacionline.org). However, when we defined IgE sensitization to allergen molecules based on a higher cutoff of more than 3.5 ISU-E, statistically significant positive associations of air pollution exposure at the birth address with sensitization against *Phleum pratense* 1 (Phl p 1) and *Felis domesticus* 1 (Fel d 1) were seen (adjusted meta-analysis OR = 3.33 [95% CI: 1.40-7.94] and adjusted meta-analysis OR = 4.98 [1.59-15.60],

TABLE III. Meta-analyses of the associations of air pollution exposure at birth or at the time of biosampling with levels of identified risk molecules (>3.5 ISU-E) measured at age 15 to 16 years in the BAMSE and GINIplus cohorts

	Bet v 1 (birch)			Phl p 1 (grass)			Fel d 1 (cat)		
Air polution indicator	**OR**	**95% CI**		**OR**	**95% CI**		**OR**	**95% CI**	
At birth									
Cases (no./total no.)		176 of 1021			203 of 1021			95 of 1021	
NO_2	1.14	0.22	5.83	1.20	0.58	2.49	1.19	0.46	3.07
NO_x	0.84	0.24	2.96	1.10	0.67	1.80	1.00	0.57	1.76
$PM_{2.5}$	1.96	0.37	10.36	**3.33**	**1.40**	**7.94**	**4.98**	**1.59**	**15.60**
$PM_{2.5}$ absorbance	0.80	0.003	234.25	2.05	0.17	24.08	3.01	0.12	73.18
PM_{10}	0.86	0.30	2.43	1.27	0.73	2.20	1.36	0.64	2.87
PM coarse	0.37	0.03	4.22	0.82	0.34	1.94	0.84	0.32	2.23
At time of biosampling									
Cases (no./total no.)		165 of 933			190 of 933			90 of 933	
NO_2	0.64	0.24	1.72	0.84	0.59	1.18	0.81	0.51	1.30
NO_x	0.56	0.16	2.03	0.91	0.67	1.23	0.85	0.56	1.30
$PM_{2.5}$	0.90	0.35	2.26	1.15	0.52	2.55	1.43	0.30	6.87
$PM_{2.5}$ absorbance	0.51	0.08	3.28	0.90	0.29	2.81	1.04	0.28	3.93
PM_{10}	0.55	0.11	2.66	0.87	0.53	1.42	1.05	0.56	1.95
PM coarse	0.44	0.06	3.35	0.93	0.65	1.31	0.84	0.34	2.11

Adjusted for sex, maternal smoking during pregnancy, anyone smoking at the child's home, breast-feeding, atopic parents, parental education, mold at home, furred pets at home, older siblings, gas cooking, study arm (GINIplus cohort), and region (for the BAMSE cohort only). Combined ORs and 95% CIs derived by random effects methods. Effects are presented for increments of 10 $\mu g/m^3$ (NO_2, PM_{10}), 1×10^{-5}/m ($PM_{2.5}$ absorbance), 5 $\mu g/m^3$ ($PM_{2.5}$, PM_{coarse}), and 20 $\mu g/m^3$ (NO_x). Boldface indicates statistical significance.

respectively, for a-5 $\mu g/m^3$ increase in $PM_{2.5}$ exposure [Table III and see Table E16 in the Online Repository at jacionline.org]). No similar associations with exposure at the time of biosampling were detected. We were not able to run corresponding analysis with sensitization to the fourth risk molecule, the major peanut allergen molecule *Arachis hypogea* 1, because of its low prevalence in the present study sample.

DISCUSSION

The present study constitutes a major extension of our previous collaborative project to examine the impact of outdoor air pollution exposure on the prevalence of allergic sensitization to common inhalant and/or food allergens in children and adolescents who were followed from birth until 16 years of age. The combined results from the 4 birth cohort studies indicated that air pollution exposure was generally not associated with IgE sensitization. However, analyses based on specific IgE to allergen extracts suggest increased risks of sensitization to birch in relation to several air pollution indicators. Further, higher air pollution exposure at birth address appeared to be associated with elevated levels of the grass allergen molecule Phl p 1, as well as with the cat allergen molecule Fel d 1 in a subset of the study population with available data on IgE sensitization against allergen molecules.

We observed no overall association between exposure to the studied air pollution components and any allergic sensitization up to adolescent age, which is in line with our previous meta-analysis based on the same cohorts exploiting data from 4- and 8-year follow-ups.[14] Similarly, recent longitudinal analysis of LISA/GINI cohorts followed for 10 years did not find consistent evidence that air pollution exposure is associated with a higher risk of sensitization in later childhood.[13] In the analyses based on specific IgE to allergen extracts, however, we found higher risks of sensitization to birch pollen in relation to exposure at the time of biosampling. Even though the observed associations appeared to be sensitive to multiple testing adjustment, we would like to acknowledge a consistent direction of positive association of sensitization against birch pollen extracts with estimated exposures at the time of biosampling to all considered pollutants, supporting the biologic plausibility of such associations. Notably, the strength of the observed association was moderate (eg, for sensitization to birch allergen extract, OR = 1.12 [95% CI = 1.01-1.25] per 10-$\mu g/m^3$ increase in NO_2 exposure); however, because air pollution exposures affect large parts of the population, even a moderately increased risk estimate may lead to a high number of extra cases resulting from this particular exposure. Some of the earlier published cohort-specific results indicated that air pollution exposure was related to pollen sensitization at age 4 to 6 years in the BAMSE cohort (OR = 1.83 [95% CI =1.02-3.28] per 46.7-$\mu g/m^3$ increase in NO_x exposure during infancy)[8] and LISA/GINI cohorts (OR = 1.45 [95% CI = 1.21-1.74] per 1.5-$\mu g/m^3$ increase in $PM_{2.5}$ exposure),[30] whereas in the PIAMA cohort associations between air pollution exposure to $PM_{2.5}$, soot, and NO_2 at birth addresses and specific sensitization were limited to food allergens.[11] In contrast, no association between long-term air pollution exposure and sensitization to any allergen was seen in the PIAMA cohort at the age of 8 years,[12] or in 9- to 10-year-old children in Oslo, Norway.[10]Another study by Janssen et al also found a positive association between NO_2 and sensitization to inhalant allergens (OR = 1.70 [95% CI = 1.03-2.81] per 17.6-$\mu g/m^3$ increase in NO_2) among Dutch children 7 to 12 years of age.[31]

There is limited evidence on the association between air pollution exposure and allergic sensitization in children beyond primary school age. Recent longitudinal analysis of LISA/GINI cohorts followed for 10 years did not find consistent evidence that air pollution exposure is associated with a higher risk of sensitization in later childhood.[13] One study from the United States demonstrated that prenatal air pollution exposure was associated higher total IgE measured at early adolescent age (median age of 12 years).[32] However, this study considered only total IgE data without investigating specific IgE to allergen extracts.

We found a statistically significant positive association of air pollution exposure at birth address with sensitization at the age of 16 years against the major grass allergen Phl p 1, as well as against the major cat allergen molecule Fel d 1, which have been recognized as the allergen molecules capable of predicting development of respiratory allergies at later ages.[20,33] As no previous study has examined IgE sensitization using allergen molecules in relation to air pollution exposure, our results represent a novel finding. It should also be noted that these analyses were limited to a subsample of individuals from 2 participating cohorts and that this observation needs to be confirmed in future larger studies.

It has been suggested that air pollution may increase sensitivity of the airway epithelium to inhalant allergens. Our findings of elevated risk for sensitization against pollen allergens are also consistent with experimental data demonstrating that inhalation of traffic-related air pollutants enhances the immune responses to airborne allergens, such as pollens.[6,34] It has been shown that certain pollen allergens such as the major birch pollen allergen *Betula verrucosa* 1 and the major grass pollen allergen *Lolium perenne* 1 can bind to respirable small particles, which may enhance the induction of respiratory allergy.[35,36] However, pollutants may also contribute to increased allergic sensitization by other mechanisms. For example, it has been demonstrated that air pollution exposure strongly upregulates the expression of grass pollen allergens and may thus increase allergen loads during the pollen season.[37] The finding that nitration of the major birch pollen allergen *Betula verrucosa* 1 enhances its immunogenicity and allergenic potential provides yet another possible mechanism by which pollution may increase allergic sensitization.[38] This possibility is further supported by the altered morphology of pollen in polluted areas.[39]

Our study has several strengths. To our knowledge, it is one of the first to assess effects of air pollution exposure on the development of allergic sensitization throughout childhood up to adolescent age based on combined analyses of prospective birth cohort studies with a total sample size of more than 6000 individuals and use of a meta-analytic approach. The 4 birth cohorts included in this study were specifically designed to examine the development of allergic diseases during childhood. Participation in the European collaborative projects ESCAPE and MeDALL provided a unique opportunity to utilize standardized individual-level exposure data generated from dedicated monitoring campaigns and area-specific LUR modeling, along with repeatedly measured outcome and confounder data harmonized across all the included cohorts. In addition, high-throughput MeDALL microarray measurements in 2 of the participating cohorts enabled investigation of IgE sensitization against allergen molecules in relation to air pollution exposure.

We assigned the exposure levels to the birth addresses and the addresses at which children resided at the time of biosampling to investigate potential critical time periods of susceptibility to air pollution. One limitation related to exposure assessment is that we used exposure models based on air pollution measurement campaigns from 2008-2010 to assess exposure to air pollution for the entire duration of follow-up. Although the measurement campaigns concurred with the most recent follow-ups of the cohorts, it may not be optimal for estimation of historical exposures. However, we have extensively investigated this issue in our previous publications based on the same data by performing sensitivity analyses using modeled concentrations of considered pollutants that were extrapolated back in time, and this did not have any influence on the results.[3,14,40] Furthermore, several validation studies have investigated the rationale of using LUR models developed from current data to estimate exposure back in time by comparing modeled and measured data. For instance, a Dutch study demonstrated that a LUR model developed from 2007 data could explain almost 80% of the variability in NO_2 concentrations measured in 1999-2000.[41] Similar observations were made in other geographic areas (ie, Vancouver, Rome, and Oslo),[42-44] thus justifying the use of LUR models developed from 2008-2010 measurement data as valid tools to assess the spatial variation of air pollution levels at earlier time points. By using spatial LUR we did not take into account long-term trends in air pollution concentrations. Previous studies have shown decreasing trends for several air pollutants, including NO_2 and $PM_{2.5}$ in the Stockholm area (BAMSE cohort) during the period from 1999 to 2009, but not in the other study areas.[45] This change might have biased associations with recent air pollution exposures, but it is less of a concern for analyses with early life exposures. Further potential limitation of the exposure assessment is that LUR models were applied only to the residential addresses, which may lead to exposure misclassification, as children spend part of their time at day care and school. However, previous studies have demonstrated high correlation between estimated air pollution exposure levels based on residential addresses only and those accounting for multiple locations, possibly on account of the fact that day care centers and primary schools are often located within the same neighborhood.[8,46] Further, the modeled individual concentrations account only for outdoor air pollution and, therefore, may not fully reflect personal exposure. A recent study in The Netherlands, Finland, and Spain compared pollutant estimates from the ESCAPE LUR models against measurements from personal monitors, showing good agreement between modeled and measured concentrations of $PM_{2.5}$ absorbance (coefficient of determination $r^2 = 0.83$), NO_2 ($r^2 = 0.79$), and NO_x ($r^2 = 0.54$).[47] Although some misclassification of true individual exposure may affect our results, the assessments of both exposure and disease were done independently from one another, thus making potential misclassification likely to be nondifferential. Further, we have adjusted for a wide set of potential confounders; however, as in most epidemiologic studies, the possibility of residual confounding cannot be ruled out. Unfortunately, we do not have empiric data on pollen counts to check potential differences in pollen exposure levels between the specific study areas.

In conclusion, the results of this study based on the data from 4 birth cohorts did not provide consistent evidence of an association between air pollution exposure and overall allergic sensitization in children up to 16 years of age. However, analyses of specific IgE to allergen extracts suggest higher risks of sensitization to birch pollen in relation to several air pollution indicators, as well as to the grass allergen molecule Phl p 1 and the cat allergen molecule Fel d 1, defined by a higher threshold of more than 3.5 ISU-E, in relation to $PM_{2.5}$ exposure at birth.

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Key messages

- **The associations of residential exposure to air pollution with IgE sensitization to common inhalant and food allergens were studied in combined analyses of 4 European birth cohorts with a standardized exposure assessment following a common protocol.**
- **Overall, the analysis results did not provide clear evidence of an association between air pollution exposure and development of IgE sensitization to common inhalant or food allergens in children up to 16 years of age.**
- **However, analyses based on specific IgE to allergen extracts suggested increased odds of sensitization to birch, as well as to the grass allergen component molecule Phl p 1 and the cat allergen Fel d 1 (defined by a higher threshold of more than 3.5 ISU-E) in relation to air pollution.**

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