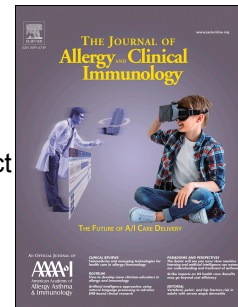


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Air pollution and IgE sensitization in four European birth cohorts - the MeDALL project

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Air pollution and IgE sensitization in four European birth cohorts - the MeDALL project

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Abstract

Background: Whether long-term air pollution exposure has effects on allergic sensitization is controversial.

Objective: To investigate associations of air pollution exposure at birth and at the time of later biosampling, with IgE sensitization against common food/inhalant allergens, or specific allergen molecules, in children up to 16 years.

Methods: Up to 6163 children from four European birth cohorts participating in the MeDALL Consortium were included in this meta-analysis: BAMSE (Sweden), LISA/GINIplus (Germany), and PIAMA (The Netherlands). Individual residential outdoor levels of particulate matter with aerodynamic diameters <2.5 , <10 , $2.5-10 \mu\text{m}$, $\text{PM}_{2.5}$ absorbance, nitrogen oxides were modelled by land-use regression. Blood samples drawn at 4-6 (n=5989), 8-10 (n=6603) and 15-16 (n=5825) years were analyzed for IgE sensitization to allergen extracts by ImmunoCAP. Additionally, IgE against 132 allergen molecules was measured using the MedALL microarray chip (n=1021).

Results: Air pollution was not consistently associated with IgE sensitization to any common allergen extract up to 16 years. However, allergen-specific analyses suggested increased risks of sensitization to birch: $\text{OR}=1.12$; 95% CI: 1.01–1.25 per $10 \mu\text{g}/\text{m}^3$ increase in NO_2 exposure. In a sub-population with microarray data, IgE to the major timothy grass allergen Phl_p_1 and cat allergen Fel_d_1 >3.5 ISU-E were related to $\text{PM}_{2.5}$ exposure at birth ($\text{OR}=3.33$; 1.40-7.94, and 4.98; 1.59-15.60, respectively, per $5 \mu\text{g}/\text{m}^3$ 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.

Key messages:

- We studied the associations of residential exposure to air pollution with IgE sensitization to common inhalant and food allergens in combined analyses of four European birth cohorts with a standardized exposure assessment following a common protocol
- Overall, our results do 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 cat allergen Fel d 1 (defined by a higher threshold of >3.5 ISU-E) in relation to air pollution.

Capsule summary:

Exposure to ambient air pollution does not increase the overall risk of sensitization to inhalant and food allergens, but IgE sensitization to birch and major grass pollen allergens may be related to air pollutants.

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

Abbreviations:

BAMSE: Children, Allergy, Milieu, Stockholm, Epidemiology

ESCAPE: European Study of Cohorts for Air Pollution Effects

GINIplus: German Infant Study on the influence of Nutrition Intervention PLUS

environmental and genetic influences on allergy development

LISA: Influences of Lifestyle Related Factors on the Human Immune System and

Development of Allergies in Childhood

LUR: Land use regression

MeDALL: The Mechanisms of the Development of ALLergy

NO₂: Nitrogen dioxide

NO_x: Nitrogen oxides

PIAMA: Prevention and incidence of asthma and mite allergy

PM_{2.5}: mass concentration of particles less than 2.5 µm in size

PM₁₀: 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

IgE: Immunoglobulin E

OR: Odds ratio

CI: Confidence interval

Introduction

Associations between outdoor air pollution exposure 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 due to 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 are 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 harmonised exposure and health data from five European cohorts (including the four cohorts participating in this analysis), we found no apparent association between air pollution exposure and allergic sensitization in children neither at 4, nor at 8 years of age.¹⁴ Recent narrative and systematic reviews evaluating the body of evidence on air pollution exposure and allergy outcomes 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 specific IgE sensitization prevalence measured only up to school age. In a longitudinal analysis of two German birth cohorts followed for 10 years, no consistent evidence that air pollution exposure increases the risk of aeroallergen sensitization in later childhood was found.¹³ To our knowledge, no combined longitudinal analysis has yet been performed on air pollution exposure and prevalence of IgE sensitization against common allergens, as well as defined allergen molecules up to 16 years of age based on prospective birth cohort studies. Therefore, we conducted the present study in the framework of the European collaborative Mechanisms of the Development of ALLergy (MeDALL)¹⁸ project aiming at evaluating the

association of air pollution exposure 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 four European birth cohorts, and applied uniform exposure assessment methodology developed within the ESCAPE (European Study of Cohorts for Air Pollution Effects, <http://www.escapeproject.eu>) project.¹⁹ Furthermore, the availability of MeDALL microarray data on IgE against 132 allergen molecules²⁰ in two of the cohorts (BAMSE and 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.

Materials and Methods

The Methods section in this article's Online Repository provides additional details on the study populations, air pollution exposure assessment, IgE microarray measurement and statistical analyses employed in this study.

Study populations

Data from four European birth cohorts participating in the MeDALL project were included in the current study: BAMSE (Sweden), PIAMA (The Netherlands), LISA and GINIplus (Germany). Participants were recruited in 1994–1999. Detailed descriptions of study design, enrollment and procedures for data collection in each cohort have been described elsewhere.^{21–23}

Air pollution exposure assessment

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

Measurement of IgE sensitization

At the age of 4 years (6 – for the LISA/GINI cohorts), 8 years (10 - for the LISA/GINI cohorts) and 16 years (15 - 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) using the ImmunoCAP System (Thermo Fisher/Phadia AB, Uppsala, Sweden), or equivalent test systems (i.e., 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 and grass pollen for all cohorts, and mugwort for all cohorts except PIAMA (Table 1). The panel of food allergen sources includes cow's milk and egg for all cohorts, and soy bean, peanut, cod fish, 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 1. Sensitization was defined as an IgE antibody level $\geq 0.35 \text{ kU}_A/\text{L}$ 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 16 years ($n=743$), as well as in GINIplus at the age of 15 years ($n=278$) with an allergen chip based on ISAC technology (Thermo Fisher) developed within

the MeDALL project.²⁶ Serum aliquots of 30 µL were incubated on the microarray for 2 hours at room temperature, and slides were washed and then incubated with fluorescence-labeled anti-IgE antibodies (Thermo Fisher) for 30 minutes. 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 two cohorts are presented in the online supplement (Table E1 in the Online Repository). The cut-off for IgE detection was set at 0.3 ISAC standardized units (ISU-E).

Statistical analyses

Associations of outdoor air pollution exposure with prevalence of sensitization until 16 years of age were assessed in each cohort separately using generalized estimating equation (GEE) models with 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 a participant lived at the time of biosampling. The models incorporated interaction terms between time indicator variable (age) and exposure to evaluate age-specific effects of exposure.

Adjusted cohort-specific ORs and 95% confidence intervals were then meta-analyzed using a random effects model,²⁷ taking into account possible nonrandom variability within and between cohorts. Statistical heterogeneity among studies was evaluated using the I^2 statistics.²⁸ A P-value <0.05 was considered significant. The results of microarray-wide analyses were adjusted for multiple testing using Bonferroni correction applied to 132 tests.²⁹ All analyses were performed with STATA Release 13.1 (StataCorp, College Station, Tx, USA).

Results

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 in the Online Repository. The distribution of potential risk factors at 4-6, 8-10 and 15-16 years in each cohort is presented in Tables E3, E4 and E5, respectively, in the Online Repository. Noteworthy, in the subset of children with IgE measurements in the BAMSE cohort, the proportion of children with allergic heredity was lower compared to the other cohorts. This is partly explained by differences in study designs between 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 where the BAMSE cohort is based. Median air pollution levels ranged from 12.4 $\mu\text{g}/\text{m}^3$ (BAMSE) to 23.2 $\mu\text{g}/\text{m}^3$ (LISA/GINI North) for NO_2 , and from 8.1 $\mu\text{g}/\text{m}^3$ (BAMSE) to 17.2 $\mu\text{g}/\text{m}^3$ (LISA/GINI North) for $\text{PM}_{2.5}$, whereas the concentrations of $\text{PM}_{\text{coarse}}$ 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 in the Online Repository. 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 (Supplementary Fig E1 in the Online Repository). The strongest correlations were observed for the LISA/GINI North cohort ($r=0.59\text{--}0.77$ for the 15-year addresses) and lowest for the BAMSE cohort ($r=0.35\text{--}0.45$ for the 16-year addresses).

In the four cohorts, the prevalence of sensitization to a combination of common inhalant and food allergen extracts ranged from 24.1% to 40.4% at the age of 4-6 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 8-10 years (in the BAMSE and LISA/GINI South cohorts, respectively), and from 41.8% to 51.2% at the age of 15-16 years (in the LISA/GINI North and LISA/GINI South cohorts, respectively), as shown in Table E7 in the Online Repository.

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 15-16 years are shown in Fig 2 and Fig E2 in the Online Repository, 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 15–16 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 odds ratios for air pollution exposure at birth ranged from OR=0.99; (95% confidence interval: 0.83–1.17), for a $10 \mu\text{g}/\text{m}^3$ increase in PM_{10} exposure to 1.32 (0.95 – 1.84), for a $5 \mu\text{g}/\text{m}^3$ increase in $\text{PM}_{2.5}$ exposure. Similar results were obtained in relation to air pollution exposure at the time of biosampling (Fig E2 in the Online Repository). The results of the fully adjusted analyses were largely comparable to those based on the basic model (Table E8 in the Online Repository). Further, increasing cut-offs for IgE positivity to any of the tested inhalant and/or food allergen extracts (ie, $\geq 0.70 \text{ kU}_\text{A}/\text{L}$ and $\geq 3.5 \text{ kU}_\text{A}/\text{L}$) had no major impact on the overall results (Table E9 in the Online Repository). Based on the analyses with exposure–age interaction terms, we did not find any indication of differences in age-specific associations neither with exposure at birth, nor with exposure at the time of biosampling (Table E10 in the Online Repository). 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 corresponding confidence intervals largely overlapped (Tables E11 and E12 in the Online Repository). However, by looking separately at associations with sensitization against single allergen extracts we observed a 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; 1.01 - 1.25, for 10 $\mu\text{g}/\text{m}^3$ increase in NO_2 exposure; OR=1.24; 1.03 - 1.50, for 10 $\mu\text{g}/\text{m}^3$ increase in PM_{10} exposure, and OR=1.23; 1.07 - 1.40, for 5 $\mu\text{g}/\text{m}^3$ increase in $\text{PM}_{\text{coarse}}$ exposure; see Tables 2, E13 in the Online Repository). No consistent association was found with sensitization against timothy grass pollen extract (Table E14 in the Online Repository).

Further, we evaluated the association of air pollution exposure with IgE sensitization to micro-arrayed allergen molecules at the age of 15-16 years 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 correction (data not shown). In the candidate analysis limited to four “risk molecules” previously linked to the risk of respiratory allergy,²⁰ we observed positive associations of sensitization against these molecules with $\text{PM}_{2.5}$ exposure at birth address that did not reach the level of statistical significance (Table E15 in the Online Repository). However, when we defined IgE sensitization to allergen molecules based on a higher cut-off of >3.5 ISU-E, statistically significant positive associations of air pollution exposure at the birth address with sensitization against Phl p 1 and Fel d 1 were seen (adjusted meta-analysis OR=3.33; 95% CI: 1.40 – 7.94, and 4.98; 1.59 - 15.60, respectively, for a 5 $\mu\text{g}/\text{m}^3$ increase in $\text{PM}_{2.5}$ exposure (Tables 3 and E16 in the Online Repository). No similar associations were detected with exposure at the time of biosampling. We were not able to run corresponding analysis with sensitization to the fourth “risk molecule”, the major peanut allergen molecule Ara h 1 due to 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 followed from birth until 16 years of age. The combined results from the four 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 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-years 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 biological plausibility of such associations. Noteworthy, the strength of the observed association was moderate, e.g. sensitization to birch allergen extract: OR=1.12; 95% CI: 1.01–1.25 per 10 µg/m³ increase in NO₂ exposure, however, since air pollution exposures affect large parts of the population,

even a moderately increased risk estimate may lead to a high number of extra cases due to this particular exposure. Some of the earlier published cohort-specific results indicated that air pollution exposure was related to pollen sensitization at 4-6 years in the BAMSE cohort (OR = 1.83; 95% CI 1.02 - 3.28, per 46.7 $\mu\text{g}/\text{m}^3$ increase in NO_x exposure during infancy),⁸ LISA/GINI cohorts (OR = 1.45; 95% CI 1.21 - 1.74, per 1.5 $\mu\text{g}/\text{m}^3$ increase in $\text{PM}_{2.5}$ exposure),³⁰ while in the PIAMA cohort associations between air pollution exposure to $\text{PM}_{2.5}$, 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 9–10-year-old children in Oslo, Norway.³¹ 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 $\mu\text{g}/\text{m}^3$ increase in NO_2) among Dutch children 7-12 years of age.³²

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 US demonstrated that prenatal air pollution exposure was associated higher total IgE measured at early adolescent age (median age of 12 years).³³ However, this study only considered 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 a major grass allergen Phl p 1, as well as against the major cat allergen molecule Fel d 1 that have been recognized as the allergen molecules being able to predict development of respiratory allergies at later ages.^{20, 34} 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 two participating cohorts and that this observation needs to be confirmed in future larger studies.

It has been suggested that air pollution may increase the sensitivity of the airway epithelium to inhalant allergens. Our findings of elevated risk for sensitization against pollen allergens are also consistent with experimental data that have demonstrated that inhalation of traffic-related air pollutants enhances the immune responses to airborne allergens, such as pollens.^{35,}

³⁶ It has been shown that certain pollen allergens such as the major birch pollen allergen Bet v 1 and the major grass pollen allergen Lol p 1 can bind to respirable small particles which may enhance the induction of respiratory allergy.^{37, 38} However, pollutants may contribute also by other mechanisms to increased allergic sensitization. For example, it has been demonstrated that air pollution exposure strongly upregulates the expression of grass pollen allergens and thus may increase allergen loads during the pollen season.³⁹ The finding that nitration of the major birch pollen allergen Bet v 1 enhances its immunogenicity and allergenic potential provides yet another possible mechanism how pollution may increase allergic sensitization.⁴⁰ This is further supported by the altered morphology of pollen in polluted areas.⁴¹

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, using a meta-analytic approach. The four birth cohorts included in this study were specifically designed to study 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 modelling, along with repeatedly measured outcome and confounder data harmonized across all the included cohorts. In addition, high-throughput MeDALL microarray measurements in two 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 those where children resided at the time of biosampling in order 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 extrapolated back in time modeled concentrations of considered pollutants, and this did not have any influence on the results.^{3, 14, 42} Furthermore, several validation studies have investigated the rationale of using LUR models developed from current data to estimate exposure back in time by comparing modelled 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 NO₂ concentrations measured in 1999–2000.⁴³ Similar observations were made in other geographical areas, i.e. Vancouver, Rome, and Oslo,^{44–46} thus, justifying the use of LUR models developed from 2008–2010 measurement data as a valid tool 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 NO₂ and PM_{2.5} in the Stockholm area (BAMSE cohort) during 1999 - 2009, but not the other study areas.⁴⁷ This change might have biased associations with recent air pollution exposures, but is less of a concern for analyses with early life exposures. Further potential limitation of the exposure assessment is that LUR models were only applied 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 due to the fact that daycares and primary schools are often located within the same neighbourhood.^{8, 48} 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 modelled and measured concentrations of PM_{2.5} absorbance (coefficient of determination, $r^2=0.83$), NO₂ ($r^2=0.79$) and NO_x ($r^2=0.54$).⁴⁹ Although some misclassification of true individual exposure may affect our results, the assessment of both exposure and disease was done independently from one another, therefore, making potential misclassification likely to be non-differential. Further, we have adjusted for a wide set of potential confounders, however, as in most epidemiological studies, the possibility of residual confounding cannot be ruled out. Unfortunately, we do not have empirical 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 four 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 cat allergen molecule Fel d 1, defined by a higher threshold of >3.5 ISU-E, in relation to PM_{2.5} exposure at birth.

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495 **Table 1. Overview of the tested allergen sources in the four birth cohorts**

Cohort	Test system	Tested allergens
BAMSE	ImmunoCAP System, Thermo Fisher/Phadia AB, Uppsala, Sweden (Phadiatop/fx5)	Inhalant allergen sources: • Outdoor: birch, timothy, mugwort • Indoor: cat, dog, mold [<i>Cladosporium herbarum</i>], 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, <i>Alternaria alternata</i>, 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, mugwort • Indoor: cat, dog, mold [<i>Cladosporium herbarum</i>], house dust mite [Der p] Food allergen sources: cow's milk, egg white, soy bean, peanut, cod fish, rye and wheat

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Table 2. 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 16 years in four European birth cohorts.

		Birch pollen extract			Timothy grass pollen extract		
		OR	95% CI		OR	95% CI	
Birth address							
Cases / Total	1494 / 6163			1965 / 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} abs	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	
Address at the time of biosampling							
Cases / Total	1409 / 5771			1842 / 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} abs	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 µg/m³ (NO₂, PM₁₀), 1×10⁻⁵ m⁻¹ (PM_{2.5} absorbance), 5 µg/m³ (PM_{2.5}, PM_{coarse}), 20 µg/m³ (NO_x). Adjusted for sex, maternal smoking during pregnancy, anyone smoking at child's home, breastfeeding, atopic parents, parental education, mold at home, furred pets at home, older siblings, gas cooking, study arm (for GINIplus cohort), and region (for the BAMSE cohort).

Table 3. 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 15-16 years in the BAMSE and GINIplus cohorts.

	Bet v 1 (birch)			Phl p 1 (grass)			Fel d 1 (cat)		
	OR	95% CI		OR	95% CI		OR	95% CI	
Birth address									
Cases / Total	176 / 1021			203 / 1021			95 / 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} abs	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
Address at the time of biosampling									
Cases / Total	165 / 933			190 / 933			90 / 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} abs	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 child's home, breastfeeding, atopic parents, parental education, mold at home, furred pets at home, older siblings, gas cooking, study arm (GINIplus cohort) and region (only for the BAMSE cohort). Combined ORs and 95% CI derived by random effects methods. Effects are presented for increments of 10 µg/m³ (NO₂, PM₁₀), 1×10⁻⁵ m⁻¹ (PM_{2.5} absorbance), 5 µg/m³ (PM_{2.5}, PM_{coarse}), 20 µg/m³ (NO_x).

FIG 1. Air pollution exposure at birth address in four European birth cohorts.

Each box contains the middle 50% of the data, with the upper edge (hinge) of the box indicating the 75th percentile and the lower edge indicating the 25th percentile (interquartile range). The line in the box represents the median. The ends of the vertical lines (“whiskers”) indicate $1.5 * IQR$.

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 in four European birth cohorts (n=6163): A) NO₂; B) NO_x; C) PM_{2.5}; D) PM_{2.5} absorbance; E) PM₁₀; F) PM_{coarse}.

Adjusted for sex, maternal smoking during pregnancy, anyone smoking at child's home, breastfeeding, atopic parents, parental education, mold at home, furred pets at home, older siblings, gas cooking, study arm (GINIplus cohort) and region (only for the BAMSE cohort). Combined ORs and 95% CI derived by random effects methods. Effects are presented for increments of $10 \mu\text{g}/\text{m}^3$ (NO₂, PM₁₀), $1 \times 10^{-5} \text{ m}^{-1}$ (PM_{2.5} absorbance), $5 \mu\text{g}/\text{m}^3$ (PM_{2.5}, PM_{coarse}), $20 \mu\text{g}/\text{m}^3$ (NO_x).

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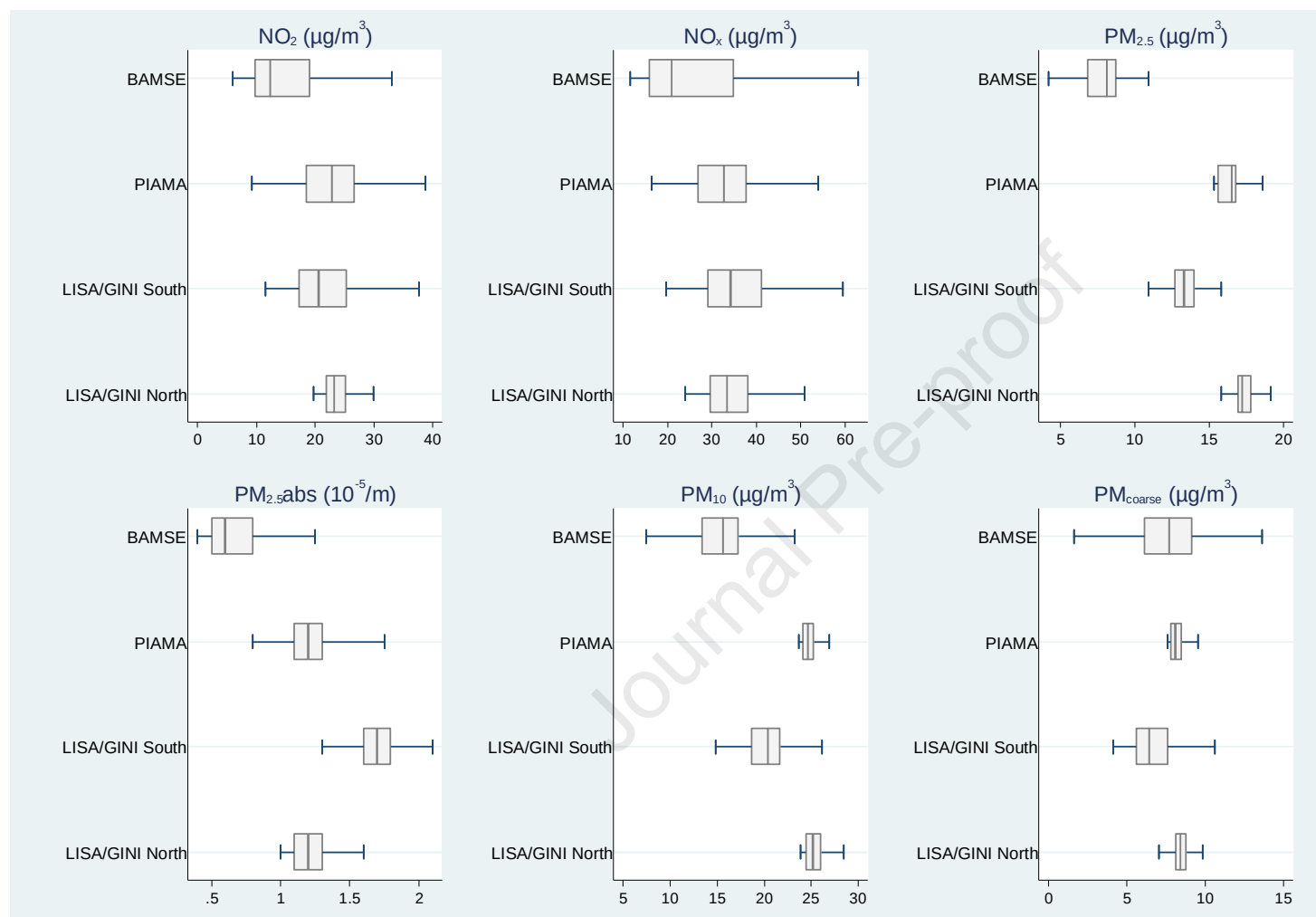
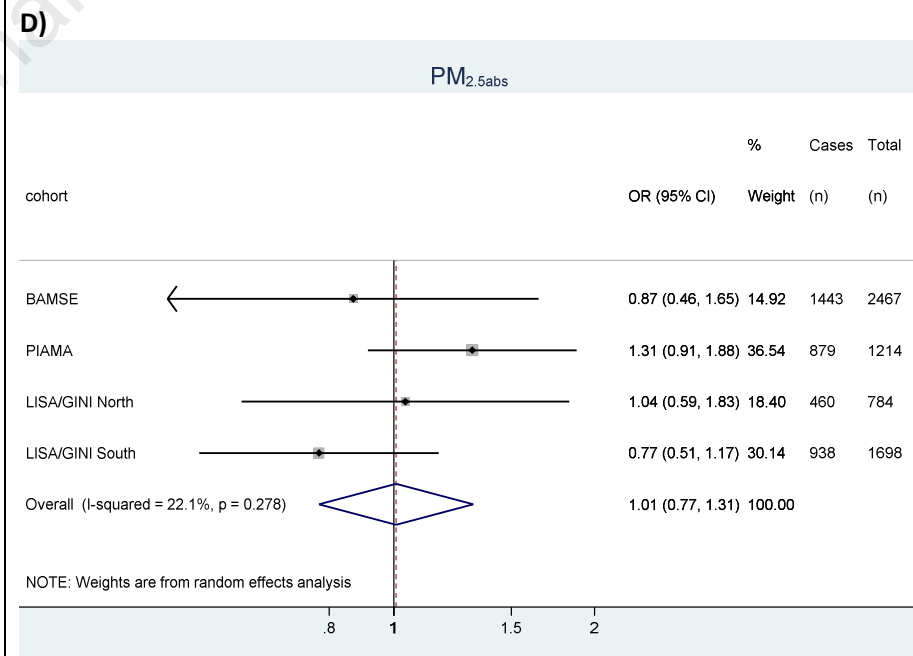
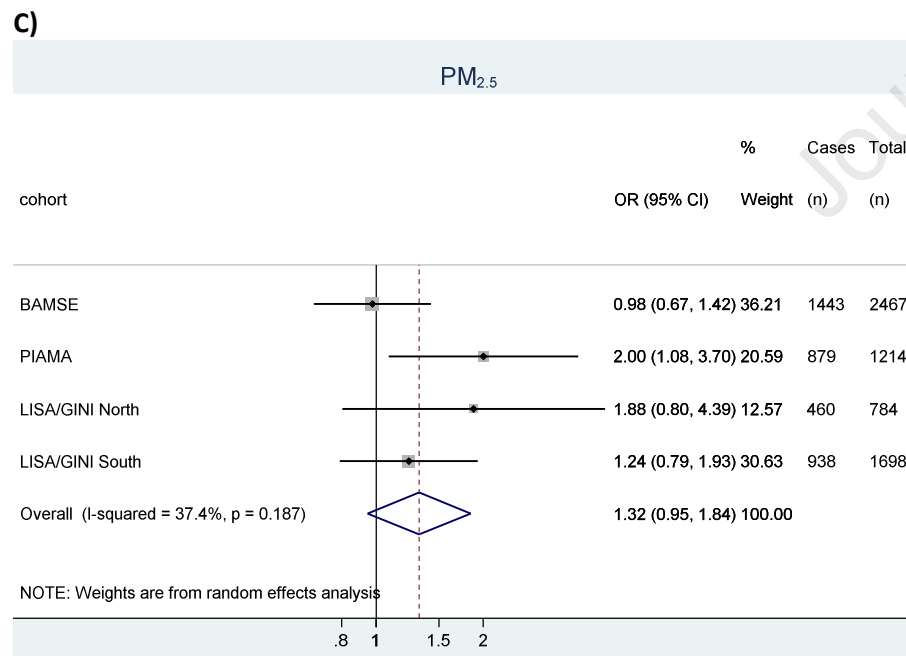
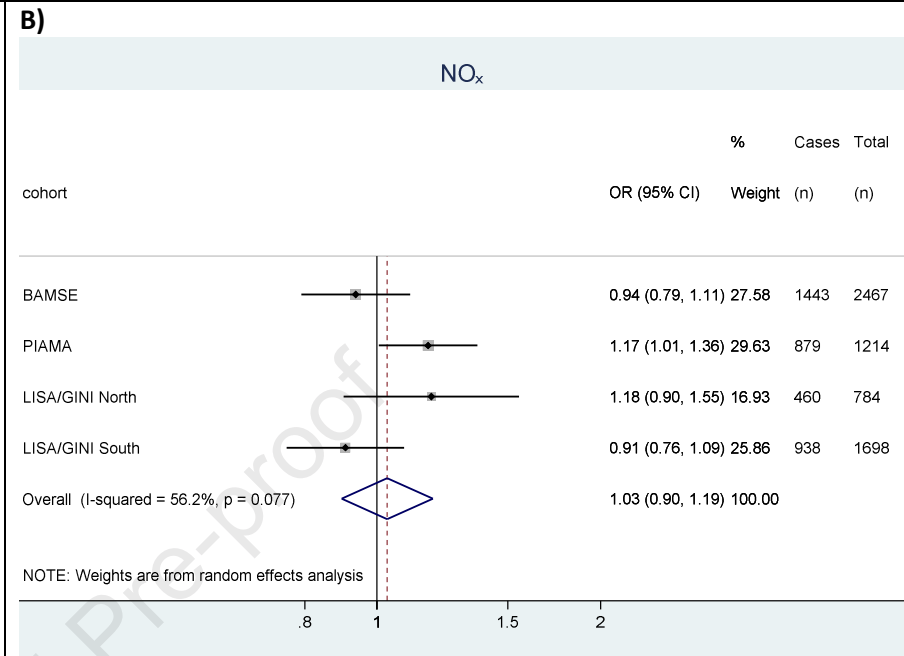
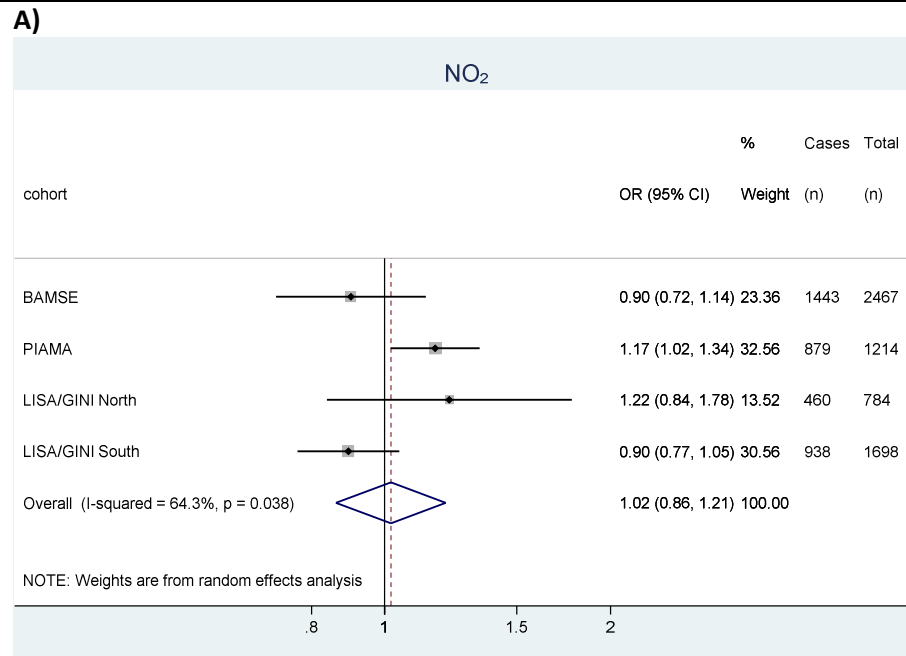


FIG 1.



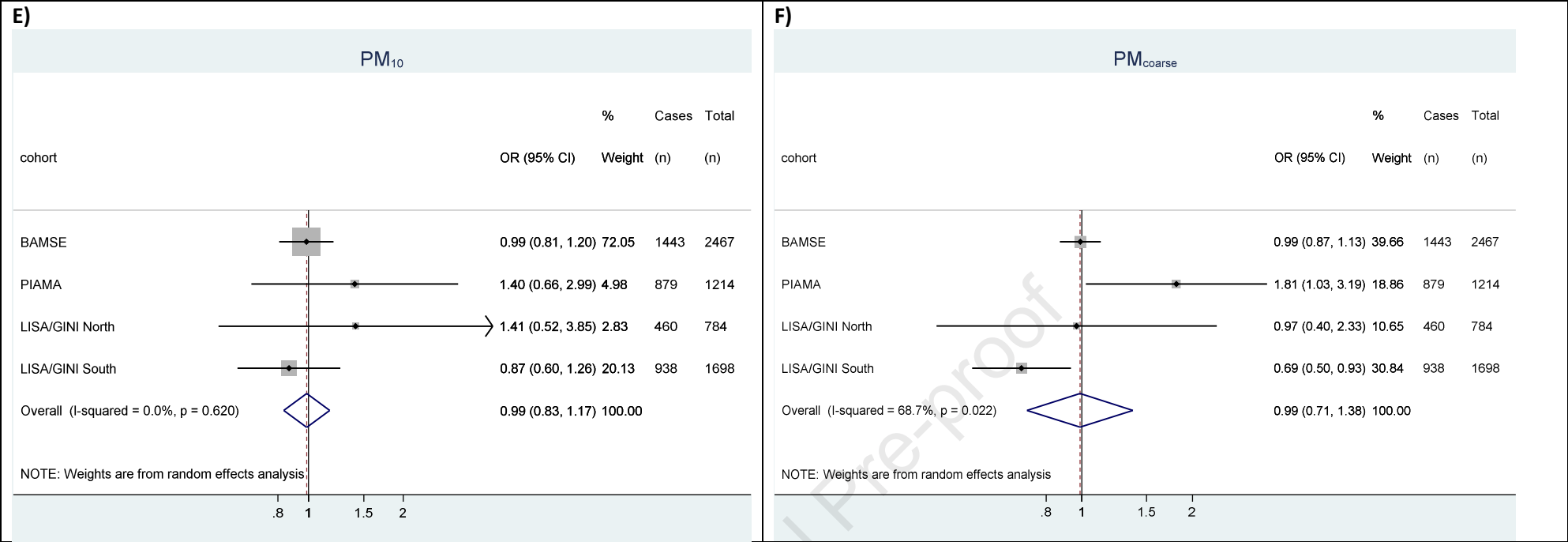


FIG 2