Rhinitis, sinusitis, and upper airway disease

Childhood allergic rhinitis, traffic-related air pollution, and variability in the *GSTP1*, *TNF*, *TLR2*, and *TLR4* genes: Results from the TAG Study

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Background: Associations between traffic-related air pollution (TRAP) and allergic rhinitis remain inconsistent, possibly because of unexplored gene-environment interactions.

Objective: In a pooled analysis of 6 birth cohorts (N_{total} = 15,299), we examined whether TRAP and genetic polymorphisms related to inflammation and oxidative stress predict allergic rhinitis and sensitization.

Methods: Allergic rhinitis was defined with a doctor diagnosis or reported symptoms at age 7 or 8 years. Associations between nitrogen dioxide, particulate matter 2.5 ($PM_{2.5}$) mass, $PM_{2.5}$

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Support for this the TAG study was provided by the AllerGen Networks of Centres of Excellence. The BAMSE study was supported by the Swedish Research Council, the Swedish Research Council FORMAS, the Swedish Heart–Lung Foundation, Stiftelsen Frimurare Barnhuset i Stockholm, the Stockholm County Council, the Swedish Environmental Protection Agency, and the Swedish Society for Medical Research. The PIAMA study is supported by The Netherlands Organization for Health Research and Development; The Netherlands Organization for Scientific Research; The Netherlands Asthma Fund; The Netherlands Ministry of Spatial Planning, Housing, and the Environment; and The Netherlands Ministry of Health, Welfare, and Sport. The GINIplus study was supported for the first 3 years by grants of the Federal Ministry for Education, Science, Research and Technology (grant 01 EE 9401-4). The 3- to 6- and 10-year follow-up examinations of the GINI study were covered from the respective budgets of the initial 4 study centers (Helmholtz Zentrum Munich [former GSF], Wesel, LMU Munich, TU Munich) and from 6 years onward in addition partly by the Federal Ministry for Environment (IUF, FKZ 20462296). The LISAplus study was supported by grants 01 EG 9732 and 01 EG 9705/2 from the Federal Ministry for Education, Science, Research and Technology; by the Federal Ministry for Environment (IUF, FKZ 20462296); and by the Helmholtz Zentrum München, Munich Center of Health. The CAPPS study was supported by the Canadian Institute of Health Research, the British Columbia Lung Association, and the Manitoba Medical Service Foundation. The SAGE study was supported by the Canadian Institute of Health Research. E. Fuertes was supported by the AllerGen Networks of Centres of Excellence (Canadian Allergy and Immune Diseases Advanced Training Initiative) and the Canadian Institutes of Health Research (Sir Frederick Banting and Charles Best Canada Graduate Scholarship). Initial discussions about the TAG collaboration took place at an AllerGen Networks of Centres of Excellence workshop "Genes and the Environment: The Genesis of Asthma and Allergy Workshop" in 2009.

Disclosure of potential conflict of interest: M. Brauer has been supported by one or more grants from and has received support for travel from the AllerGen Networks of Centres of Excellence. E. Fuertes has been supported by one or more grants from the AllerGen Networks of Centres of Excellence and has received support for travel from the Canadian Institutes of Health Research (Sir Frederick Banting and Charles Best Canada Graduate Scholarship). B. Hoffmann has consultancy arrangements with Health Effects Institut; has received one or more grants from or has one or more grants pending with the German Research Society, German Environmental Agency, EU; and has received one or more payments for lecturing from or is on the speakers' bureau for MSE class University of Mainz. S. Koletzko has been supported by a BMBF grant from the Childhood Foundation. E. MacIntyre has been supported by one or more grants from the AllerGen Networks of Centres of Excellence. G. Pershagen has been supported by one or more grants from the Swedish Research Council, Swedish Research Council FORMAS. A. von Berg has received one or more payments for lecturing from or is on the speakers' bureau for the Nestlé Nutrition Institute. T. Bellander has been supported by one or more grants from and has received support for travel from the Swedish Research Council FORMAS, the Swedish Heart-Lung Foundation, Stiftelsen Frimurare Barnhuset i Stockholm, the Stockholm County Council, the Swedish Environmental Protection Agency, and the Swedish Society for Medical Research and has received one or more grants from or has one or more grants pending with the Swedish EPA, the Swedish Research Council FORMAS, and the Swedish Transport Authority. D. Postma has consultancy arrangements with AstraZeneca, Boehringer Ingelheim, GSK, Nycomed, and Teva; has received one or more grants from or has one or more grants pending with Chiesi, GSK, and AstraZeneca; and has received one or more payments for lecturing from or is on the speakers' bureau for AstraZeneca, Chiesi, and Nycomed. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication August 30, 2012; revised February 5, 2013; accepted for publication March 6, 2013.

Available online April 30, 2013.

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0091-6749/$36.00

http://dx.doi.org/10.1016/j.jaci.2013.03.007

absorbance, and ozone, estimated for each child at the year of birth, and single nucleotide polymorphisms within the *GSTP1*, *TNF*, *TLR2*, or *TLR4* genes with allergic rhinitis and aeroallergen sensitization were examined with logistic regression. Models were stratified by genotype and interaction terms tested for gene-environment associations.
Results: Point estimates for associations between nitrogen dioxide, $PM_{2.5}$ mass, and $PM_{2.5}$ absorbance with allergic rhinitis were elevated, but only that for $PM_{2.5}$ mass was statistically significant (1.37 [1.01, 1.86] per 5 μg/m^3). This result was not robust to single-cohort exclusions. Carriers of at least 1 minor rs1800629 (*TNF*) or rs1927911 (*TLR4*) allele were consistently at an increased risk of developing allergic rhinitis (1.19 [1.00, 1.41] and 1.24 [1.01, 1.53], respectively), regardless of TRAP exposure. No evidence of gene-environment interactions was observed.
Conclusion: The generally null effect of TRAP on allergic rhinitis and aeroallergen sensitization was not modified by the studied variants in the *GSTP1*, *TNF*, *TLR2*, or *TLR4* genes. Children carrying a minor rs1800629 (*TNF*) or rs1927911 (*TLR4*) allele may be at a higher risk of allergic rhinitis.
(J Allergy Clin Immunol 2013;132:342-52.)

Key words: *Childhood allergic rhinitis, air pollution, genetics, interaction, TNF, TLR4*

Abbreviations used

APMoSPHERE:	Air Pollution Modelling for Support to Policy on Health and Environmental Risk in Europe
BAMSE:	Children, Allergy, Milieu, Stockholm, Epidemiological Survey
CAPPS:	Canadian Asthma Primary Prevention Study
GINIplus:	German Infant study on the influence of Nutritional Intervention plus environmental and genetic influences on allergy development
GSTP1:	Glutathione-*S*-transferase pi 1
LISAplus:	Lifestyle related factors, Immune System and the development of Allergies in Childhood plus the influence of traffic emissions and genetics study
LUR:	Land-use regression
NO_2:	Nitrogen dioxide
OR:	Odds ratio
PIAMA:	Prevention and Incidence of Asthma and Mite Allergy
PM:	Particulate matter
SAGE:	Study of Asthma, Genes, and Environment
SNP:	Single nucleotide polymorphism
TAG:	Traffic, Asthma, and Genetics
TLR:	Toll-like receptor
TRAP:	Traffic-related air pollution

Recent global estimates indicate that 8.5% of children aged 6 to 7 have allergic rhinitis, and the prevalence is higher among 13 to 14 year olds (14.6%).[1] The continued increase in prevalence in recent years in a majority of countries is especially concerning.[2] Allergen exposure is strongly associated with allergic rhinitis onset. Early-life factors (young maternal age, multiple gestation, and low birth weight), family history, ethnicity, and environmental factors (environmental tobacco smoke, urban living, lifestyle, nutrition, air pollution) are also believed to be important.[3-5]

Substantial experimental and toxicologic evidence of the adverse effects of traffic-related air pollution (TRAP) on allergic disease exists, and epidemiologic evidence is building,[6] as summarized in a recent review.[7] Given its association with asthma, TRAP has been investigated as a potential cause of allergic rhinitis, and several recent large studies support a positive association.[8,9] However, some studies have failed to find an association between the prevalence of allergic rhinitis symptoms and exposure to air pollution.[10-12]

Whether TRAP increases the risk of allergic disease development and exacerbates symptoms in a genetically vulnerable subgroup remains largely unknown.[7,13] Gene-environment interactions, which have been rarely considered in previous studies of allergic rhinitis, may provide some insight and have thus been recommended.[14] Many studies that examined the interplay between genetic susceptibility and TRAP on respiratory conditions have focused on genes in the oxidative stress and inflammation pathways.[15]

Genetic variants of the glutathione-*S*-transferase pi 1 (*GSTP1*) gene have sparked considerable interest, given the existence of common functional variants in the general population, the role of *GSTP1* in cellular protection against oxidative stress, and the presence of the cytosolic glutathione-*S*-transferase proteins in the human lung.[16] The evidence of a gene-environment interaction appears strongest for the Ile105Val (rs1695) single nucleotide polymorphism (SNP) within the *GSTP1* gene.[17-23] Gene-environment interactions have also been observed for the G308A (rs1800629) SNP within the *TNF* gene for passive smoke exposure and childhood asthma[24] and for ozone exposure with lung function and wheezing.[25,26] Furthermore, a gene-gene-environment interaction between the G-308A *TNF* variant, *GSTP1* variants, and nitrogen dioxide (NO_2) exposure was documented for allergic outcomes.[23] Members of the Toll-like receptor (*TLR*) family may also be important, given their key roles in controlling innate and adaptive immune responses. Genetic polymorphisms in *TLR*s have already been associated with allergic rhinitis[27] and may modify the link between particulate matter and childhood asthma.[28]

With the use of a pooled analysis that combined data from 6 birth cohorts with individual-level assessment of air pollution exposure, we examined the association among TRAP, allergic rhinitis, and aeroallergen sensitization in children and the influence of 10 SNPs related to inflammation and oxidative stress metabolism in the *GSTP1, TNF, TLR2,* and *TLR4* genes.

METHODS

Data sources

The Traffic, Asthma, and Genetics (TAG) study population is composed of 15,299 children recruited in 6 birth cohorts: the Canadian Asthma Primary Prevention Study (CAPPS),[29] the Study of Asthma, Genes, and Environment (SAGE),[30] the Children, Allergy, Milieu, Stockholm, Epidemiological Survey (BAMSE),[31,32] the Prevention and Incidence of Asthma and Mite Allergy study (PIAMA),[33] the German Infant Nutritional Intervention study (GINIplus),[34] and the Lifestyle related factors, Immune System and the development of Allergies in Childhood study (LISAplus).[35] Data on several health outcomes, environmental exposures, and covariates were collected via either parent- or self-completed questionnaires at various time points according to each cohort's respective information collection strategy. Information across cohorts was harmonized into common variables. A detailed description of this harmonization process and the recruitment and follow-up of each cohort is provided elsewhere (MacIntyre et al, submitted 2012).

Assessment of outcomes

The assessment of allergic rhinitis differed slightly across cohorts; the 2 Canadian cohorts (CAPPS and SAGE) relied on a diagnosis during an

TABLE I. Characteristics of participating cohorts

Cohort (country)	Full name of cohort	Areas included	Study type	Recruitment	Sample size*	Cohort-specific allergic rhinitis definition	Available aeroallergens
BAMSE (Sweden)	Children, Allergy, Milieu, Stockholm, Epidemiological Survey	Jarfalla, Solna, Sundbyberg, Stockholm	Population-based birth cohort with wheeze nested case-control	1994-6	982	Symptoms (sneezing, runny, or blocked nose; itchy, red, and watery eyes) after exposure to furred pets or pollen or a medical diagnosis of allergic rhinitis anytime between 4 and 8 y of age	Cat, dog, house dust mites, molds, birch
CAPPS (Canada)	Canadian Asthma Primary Prevention Study	Vancouver, Winnipeg	Randomized controlled study with asthma intervention	1995	545	Medical diagnosis of allergic rhinitis assessed at 7-y follow-up	Cat, dog, house dust mites, *Alternaria*, feathers, grass, *Cladosporium*, weeds, cockroaches, ragweed, trees
GINIplus (Germany)	German Infant Nutritional Intervention	Munich, Wesel	Population-based birth cohort; subset selected for nutritional intervention	1995-8	5991	Medical diagnosis of allergic rhinitis or hay fever during the past 12 mo (asked at 8-y follow-up)	Cat, dog, house dust mites, *Cladosporium*, birch, grass, mugwort, rye
LISAplus (Germany)	Lifestyle-related factors on the Immune System and development of Allergies in Childhood	Leipzig, Munich, Wesel	Population-based birth cohort	1997-9	3095	Medical diagnosis of allergic rhinitis or hay fever during the past 12 mo (asked at 8-y follow-up)	Cat, dog, house dust mites, *Cladosporium*, birch, grass, mugwort, rye
SAGE (Canada)	Study of Asthma Genes and the Environment	Winnipeg	Population-based cohort with asthma nested case-control	1995	723	Medical diagnosis of allergic rhinitis assessed at 8-y follow-up	Cat, dog, feathers, grass, ragweed, trees, weeds
PIAMA (The Netherlands)	Prevention and Incidence of Asthma and Mite Allergy	Communities in the northern, central, and western areas	Population-based birth cohort; subset selected for mattress cover intervention	1996-7	3963	Sneezing, runny/blocked nose during the past 12 mo (asked at 8-y follow-up)	Cat, dog, house dust mites, birch, *Dactylis*, grass, *Alternaria*

*Number of children included in the TAG database.

assessment by a physician at a follow-up visit, the 2 German cohorts (GINIplus and LISAplus) relied on the report of a doctor's diagnosis during the past 12 months, and the PIAMA and BAMSE cohorts required only the report of symptoms in the past 12 months and 5 years, respectively (Table I). The 8-year follow-up was selected as the time point of interest because information on allergic rhinitis was available for all but 1 cohort at this age. For CAPPS, the assessment was made at 7 years of age.

Sensitization was assessed by skin prick testing at 7 years of age for CAPPS and SAGE, with a positive reaction defined as having a wheal diameter of ≥3 mm. For GINIplus, LISAplus, BAMSE, and PIAMA, sensitization was assessed by measuring specific IgE levels, with a positive reaction defined as any value of 0.35 kU/L or greater (at 6 years of age for the former 2 cohorts and 8 years for the latter 2 cohorts). Birch, *Dactylis*, timothy grass, mugwort, ragweed, rye, trees, and weeds were considered as outdoor aeroallergens, and *Alternaria*, cats, *Cladosporium*, dogs, feathers, house dust mites, molds, and cockroaches were considered as indoor aeroallergens. All available allergens were included in the overall sensitization analysis. Not all cohorts had information on all allergens (Table I).

Air pollution estimates

Unique NO2 concentration estimates were available for 55.4% (8470/15,299; 6/6 cohorts) of participants' home address at the time of birth. For all cohorts except BAMSE, the NO_2 estimates were derived with land-use regression (LUR) modeling. The LUR models developed for the European cohorts (GINIplus and LISAplus [Munich city only] and PIAMA) were created as part of the Traffic Related Air Pollution and Childhood Asthma collaboration.[36,37] With the use of a similar methodology, LUR models were developed for the 2 Canadian cohorts[38,39] and for the cities of Wesel and Leipzig within the GINIplus and LISAplus cohorts.[40] NO_2 estimates for the BAMSE cohort were obtained from a dispersion model, as previously described.[41] Particulate matter 2.5 ($PM_{2.5}$) mass and $PM_{2.5}$ absorbance concentrations, calculated with the same methodology as for NO_2, are available for 38.5% (5893/15,299; 3/6 cohorts) and 56.3% (8615/15,299; 4/6 cohorts) of participants, respectively. Ozone estimates were available for 76.8% (11,757/15,299; 4/6 cohorts) of participants. These were calculated as part of the Air Pollution Modelling for Support to Policy on Health and Environmental Risk in Europe (APMoSPHERE) project for PIAMA, GINIplus, and LISAplus,[42] and with ambient monitoring network data for the CAPPS cohort, as previously described.[43] Unlike for the other pollutants, the ozone estimates were not derived with any specific traffic components; thus, they represent air pollution in general, rather than TRAP. The estimated exposures for NO_2, $PM_{2.5}$ mass, and $PM_{2.5}$ absorbance were positively correlated (r = 0.35, 0.81, 0.49 for NO_2 and $PM_{2.5}$ mass, NO_2 and $PM_{2.5}$ absorbance, and $PM_{2.5}$ mass and $PM_{2.5}$ absorbance, respectively). Ozone was negatively correlated (r = −0.25, −0.18, −0.15 for NO_2, $PM_{2.5}$ mass, and $PM_{2.5}$ absorbance, respectively).

Genotyping

In total, 47.3% (7229/15,299) of TAG participants were genotyped for at least 1 SNP of interest. For CAPPS and SAGE, genotyping was done with the Illumina BeadArray system (Illumina, San Diego, Calif). Genotyping in PIAMA was performed by the Competitive Allele-Specific PCR with the use of KASParTM genotyping chemistry (K-Biosciences, Herts, United Kingdom). For the 2 German cohorts (GINIplus and LISAplus) and the Swedish BAMSE cohort, SNPs were genotyped with the iPLEX (Sequenom, San Diego, Calif) method by means of matrix-assisted laser desorption ionization time of flight mass spectrometry method (Mass Array; Sequenom, San Diego, Calif), with the exception of rs1695 which was detected with the restriction fragment length polymorphism approach (in GINIplus and LISAplus only).[44] All SNPs had a genotyping success rate >93%.

Analytic strategy

Associations between a pollutant or an SNP and each outcome were assessed with logistic regression. The effect of each SNP on allergic rhinitis was examined in a dominant genetic model (carriers of at least 1 minor allele vs homozygous major allele carriers). Elevated risks of disease were analyzed per increase of 10 $\mu g/m^3$ for NO_2 and ozone, per increase of 5 $\mu g/m^3$ for $PM_{2.5}$ mass, and per increase of 10^{-5}/m for $PM_{2.5}$ absorbance (roughly the interquartile range of each pollutant in the pooled data). All models were adjusted for covariates selected *a priori* (city/center, cohort, sex, birth weight, parental history of atopic disease, maternal smoking during pregnancy, exposure to environmental tobacco smoke at the time of follow-up, intervention status [when applicable], and maternal age at birth). The latter variable was used as a surrogate of socioeconomic status, because women from a higher socioeconomic background tend to have children at older ages,[45] and a positive association between maternal age at birth and socioeconomic status has been observed in previous studies of similar populations.[46-48] To assess whether the relationship between a pollutant and an outcome differed by genotype, models were run separately for homozygous major allele carriers and heterozygous/homozygous minor allele carriers. Finally, gene-environment associations were examined by including interaction terms in the models.

All results are presented by cohort, except for the GINIplus and LISAplus studies, which are presented separately for Munich and Wesel/Leipzig because the measurement campaigns for the LUR modeling were conducted at different time points. Pooled results, which take advantage of the full available statistical power, are also presented. To assess the influence of each cohort on our pooled findings, we examined the results after a step-wise exclusion of each cohort. All results are presented as odds ratios (ORs) and 95% CIs. All statistical analyses were conducted with R version 2.13.1.

RESULTS

The study characteristics of the 6 participating cohorts are summarized in Table I, one cohort of which (CAPPS) only recruited children with a positive history for parental allergic disease, and 2 cohorts of which are nested case-control studies (for asthma [SAGE] and wheeze [BAMSE], respectively). After excluding children with no information on any of the air pollutants (n = 2100) or health outcomes (n = 4416), 10,023 children remained in the study and are described in Table II. However, not all children were included in all analyses because of missing covariate information. Overall, 1298 children (13.7%) had allergic rhinitis at the time of follow-up. The 2 Canadian cohorts (CAPPS and SAGE) that involved an active physician assessment at the follow-up visit had the highest rates of allergic rhinitis. The cities in the German cohorts (Munich and Wesel/Leipzig) that relied on the report of a doctor diagnosis of allergic rhinitis in the past 12 months had the lowest prevalences.

In total, 31.7% (1968/6212) of children were sensitized to at least 1 aeroallergen. Among subjects with data on allergic rhinitis and sensitization, 11.3% (637/5640) had both conditions. Among children with a doctor diagnosis of allergic rhinitis who also had available sensitization data, 68.9% (637/925) were sensitized (range per cohort is 42.7% in SAGE and 91.9% in Wesel/Leipzig). Given that approximately 30% of our subjects with allergic rhinitis were not sensitized to any tested allergen, it is likely that our disease definition includes both children with allergic and nonallergic rhinitis.

The characteristics of each SNP are presented in Table III by cohort and in the pooled data. All SNPs were in Hardy-Weinberg equilibrium with the exception of rs4891 in the *GSTP1* gene, which was excluded from the analysis. In this study, we focused on SNPs related to oxidative stress and inflammation that were available in at least 3 cohorts.

Main environmental effects

Substantial overlap was observed in the distribution of NO_2 and $PM_{2.5}$ absorbance between cohorts, but less so for ozone and $PM_{2.5}$ mass (Fig 1). In the pooled analysis, the point estimates for the association between NO_2, $PM_{2.5}$ mass, and $PM_{2.5}$ absorbance and allergic rhinitis at 7 or 8 years of age were elevated, but only that for $PM_{2.5}$ mass reached statistical significance after covariate adjustment (OR [95% CI], 1.37 [1.01-1.86] per 5 $\mu g/m^3$ $PM_{2.5}$ mass; 1.10 [0.95-1.26] per 10 $\mu g/m^3$ NO_2; 1.16 [0.96-1.41] per 10^{-5}/m $PM_{2.5}$ absorbance) (Fig 2; see also Table E1 in this article's Online Repository at www.jacionline.org). No significant associations were observed between any of the pollutants and aeroallergen sensitization in the pooled analysis. Furthermore, all associations between air pollutants and atopic allergic rhinitis (allergic rhinitis and sensitization to any allergen) were null (data not shown).

The elevated risk estimates found between allergic rhinitis and the air pollutants were heavily influenced by increased risks seen in the PIAMA cohort, as previously published[9] (eg, 1.37 [1.01-1.86] when all cohorts are included and 1.02 [0.62-1.67] when PIAMA is excluded, for the association between $PM_{2.5}$ mass and allergic rhinitis). This observation is further supported by the relatively inconsistent trend in the results seen across cohorts.

Main genetic effects

Carriers of at least 1 minor rs1800629 (1.19 [1.00-1.41]) or rs1927911 (1.24 [1.01-1.53]) allele were at increased risk of developing allergic rhinitis in the pooled analysis (Fig 3; see also Table E2 in this article's Online Repository at www.jacionline.org). When examining the cohort-specific analyses, the estimates for rs1800629 and rs1927911 were elevated in 4 of 6 and 4 of 5 cohorts, respectively. Furthermore, during the step-wise exclusion of each cohort, the pooled point estimates remained similar, although loss of statistical significance was occasionally observed (rs1800629 with SAGE, 1.19 [1.00-1.41]; rs1800629 without SAGE, 1.21 [1.01-1.46]; rs1927911 with SAGE, 1.24 [1.01-1.53]; rs1927911 without SAGE,: 1.16 [0.94-1.45]).

No significant associations were documented between any of the SNPs investigated and aeroallergen sensitization in the single cohort and pooled analyses (Table E2). These results remained unchanged when the analysis was stratified by indoor and outdoor allergens. The ORs for atopic allergic rhinitis with rs1800629 and rs1927911 were elevated but not significant. This loss of significance may be due to a drop in sample size because sensitization data were only available for a subset of the population or may reflect a true reduced effect on this outcome (allergic rhinitis, 1.19

TABLE II. The TAG study population

	BAMSE	CAPPS	Munich	Wesel/Leipzig	SAGE	PIAMA	Pooled
Characteristics							
Males, no. (%)	919 (52.8)	372 (53.8)	2784 (51.2)	2355 (51.3)	235 (56.2)	3358 (51.4)	10023 (51.6)
Parental history of allergies, no. (%)	919 (58.1)	372 (92.5)	2779 (53.9)	2354 (34.4)	235 (67.7)	3358 (50.1)	10017 (50.2)
Environmental tobacco smoke during pregnancy, no. (%)	919 (12.9)	369 (7.9)	2437 (13.4)	2060 (16.4)	227 (11.9)	3316 (16.0)	9328 (14.7)
Environmental tobacco smoke at home at 7 or 8 y of age, no. (%)	911 (17.8)	372 (18.0)	2566 (14.8)	2107 (26.8)	229 (20.5)	3238 (16.9)	9423 (18.8)
Older siblings, no. (%)	919 (49.5)	372 (54.8)	2781 (42.7)	2348 (50.4)	198 (68.2)	3351 (48.0)	9969 (47.9)
Intervention participation, no. (%)	919 (0)	372 (53.5)	2784 (31.4)	2355 (27.3)	235 (0)	3358 (17.8)	10020 (23.1)
Birth weight (g), mean ± SD	3500.2 ± 577.3	3495.5 ± 642.4	3415.2 ± 437.5	3527.0 ± 478.2	3378.6 ± 636.3	3507.2 ± 546.1	3489.3 ± 513.5
Maternal age at birth (y), mean ± SD	30.7 ± 4.5	31.8 ± 5.0	32.2 ± 4.1	30.4 ± 3.9	28.9 ± 5.3	30.3 ± 3.9	31 ± 4.1
Health outcomes							
Allergic rhinitis at age 7 or 8 follow-up, no. (%)	913 (17.9)	372 (30.1)	2606 (7.7)	2130 (6.3)	190 (40.0)	3240 (18.9)	9451 (13.7)
Sensitization to any aeroallergen, no. (%)	766 (24.8)	359 (45.1)	1668 (29.2)	1319 (26.2)	234 (37.2)	1866 (37.4)	6212 (31.7)
Sensitization to indoor aeroallergen, no. (%)	766 (20.6)	359 (36.8)	1668 (17.8)	1319 (18.3)	234 (27.8)	1712 (34.1)	6058 (24.4)
Sensitization to outdoor aeroallergen, no. (%)	762 (18.1)	358 (21.8)	1668 (21.6)	1319 (19.0)	234 (20.9)	1865 (17.4)	6206 (19.3)
Allergic rhinitis and sensitization, no. (%)	760 (14.5)	359 (20.6)	1490 (8.9)	1094 (7.2)	189 (16.9)	1748 (12.0)	5640 (11.3)
Allergic rhinitis with available sensitization data, no. (%)	143	106	148	86	75	367	925

Number indicates number of children with available data for indicated covariate/outcome and for the last row only, the number of children with allergic rhinitis who also have available sensitization data. Percentage is of children with this covariate/outcome among those with available data.

[1.00, 1.41] and 1.24 [1.01, 1.53] vs atopic allergic rhinitis, 1.13 [0.91, 1.40] and 1.13 [0.88, 1.46] for rs1800629 and rs1927911, respectively).

Genotype stratification and interaction effects

Stratified analyses did not show an increased risk of allergic rhinitis among heterozygous/homozygous minor allele carriers exposed to TRAP (Table IV). Only the association between allergic rhinitis and $PM_{2.5}$ mass among rs2737190 (*TLR4*) homozygous major allele carries was significant (2.77 [1.07-7.15]), but this association was also driven by the PIAMA cohort. All interaction terms were nonsignificant (*P* values ranged from .06 for rs10759931 by NO_2 to .99 for rs1800629 by NO_2).

For aeroallergen sensitization, all results from the stratified analyses were null (data not shown). Accordingly, all but 1 interaction term testing for gene-environment interactions for aeroallergen sensitization were nonsignificant in the pooled analyses (*P* values ranged from .03 [rs1695 by ozone] to .99 [rs2737190 by $PM_{2.5}$ absorbance]). After stratification into indoor and outdoor aeroallergen categories, a significantly elevated risk between indoor aeroallergen sensitization and NO_2 among minor rs1800629 allele carriers was observed (1.52 [1.09-2.12] per 10 $\mu g/m^3$ increase in NO_2), but no interaction was found ($P = .27$); the results for outdoor aeroallergens and this SNP were null (1.01 [0.70-1.45] per 10 $\mu g/m^3$ increase in NO_2).

DISCUSSION

The results of this large collaborative project do not suggest that TRAP increases the risk of allergic rhinitis in general. Although the estimate for $PM_{2.5}$ mass was significantly elevated, and the estimates for both NO_2 and $PM_{2.5}$ absorbance were also elevated, these results were mainly driven by only 1 cohort (PIAMA) and were not replicated in the other 5 cohorts. No associations were observed for ozone; however, the spatial scale of the APMoSPHERE model from which the ozone estimates were estimated is relatively broad (1 × 1 km) and may incorporate more exposure misclassification than estimates for the other pollutants.

In our study, we found suggestive evidence that children with at least 1 adenine at the 308 position in the *TNF* gene (rs1800629) may be at an elevated risk of allergic rhinitis at 7 or 8 years of age. To our knowledge, only 3 other studies have investigated this association. Zhu et al[49] found no association between *TNF* and the development of atopy, asthma, and rhinitis in a high-risk population of 373 infants. However, Gentile et al[50] found that among 124 infants, minor allele carriers of the *TNF* gene variant were at a higher risk of having a parental history of allergic disease. Moreover, a recent study found a strong association between the rs1800629 SNP and allergic rhinitis exacerbation in a population of 269 adult Pakistani patients.[51] Our study is the first to document this association in school-age children, and our results are based on a substantially larger sample size than those used in previous studies.

The association between the rs1800629 SNP and allergic rhinitis is biologically plausible. The rs1800629 SNP is located within the promoter region of the *TNF* gene, which is thought to affect the expression of the pleotropic proinflammatory cytokine *TNF*-α.[52,53] Elevated levels of *TNF*-α have been observed in patients with allergic rhinitis,[54,55] and studies in mice suggest that the lack of this cytokine inhibits allergic rhinitis development.[56] Functional and biological studies that elucidate the role of *TNF*-α in allergic rhinitis development are required, and future epidemiologic studies should aim to replicate our result.

TABLE III. SNP characteristics and genotype frequencies of the study population

Gene symbol	SNP	Alleles*	Location	BAMSE, no. (MAF)	CAPPS, no. (MAF)	Munich, no. (MAF)	Wesel/Leipzig, no. (MAF)	SAGE, no. (MAF)	PIAMA, no. (MAF)	Pooled, no. (MAF)
GSTP1	rs1138272	C/T	Exon (Ala114Val)	861 (9.0)	345 (7.0)	903 (9.4)	1252 (9.3)	183 (8.2)	1926 (9.3)	5470 (9.1)
	rs1695	A/G	Exon (Ile105Val)	897 (32.9)	345 (31.2)	1470 (35.0)	1194 (34.7)	181 (31.5)	1909 (36.1)	5996 (34.7)
	rs4891†	T/C	Exon (synonymous)	873 (30.5)	—	740 (49.4)	684 (45.3)	—	1949 (36.9)	4246 (39.1)
TNF	rs1800629	G/A	Promoter	854 (15.5)	346 (14.2)	823 (14.7)	1182 (17.7)	185 (13.0)	1906 (18.9)	5296 (16.9)
TLR2	rs4696480	T/A	Intron	—	—	391 (51.2)	382 (49.5)	—	912 (46.9)	1685 (48.8)
TLR4	rs10759930	C/T	Promoter	—	347 (40.2)	823 (40.3)	1182 (37.1)	—	—	2352 (38.7)
	rs10759931	G/A	Promoter	—	—	824 (40.3)	1183 (37.1)	—	891 (40.7)	2898 (39.1)
	rs10759932	T/C	Promoter	—	—	387 (13.7)	384 (13.3)	—	908 (11.9)	1679 (12.6)
	rs1927911	C/T	Intron	—	347 (26.5)	824 (25.5)	1181 (25.3)	185 (20.5)	909 (24.0)	3446 (24.9)
	rs2737190	A/G	5′ Untranslated region	—	—	823 (31.2)	1184 (32.6)	—	919 (32.2)	2926 (32.1)
	rs2770150	T/C	Promoter	—	347 (25.6)	822 (28.5)	1181 (29.1)	186 (26.9)	896 (29.1)	3432 (28.5)

MAF, Minor allele frequency.
*Major allele/minor allele.
†SNP was not in Hardy-Weinberg equilibrium and subsequently was eliminated from the analysis.

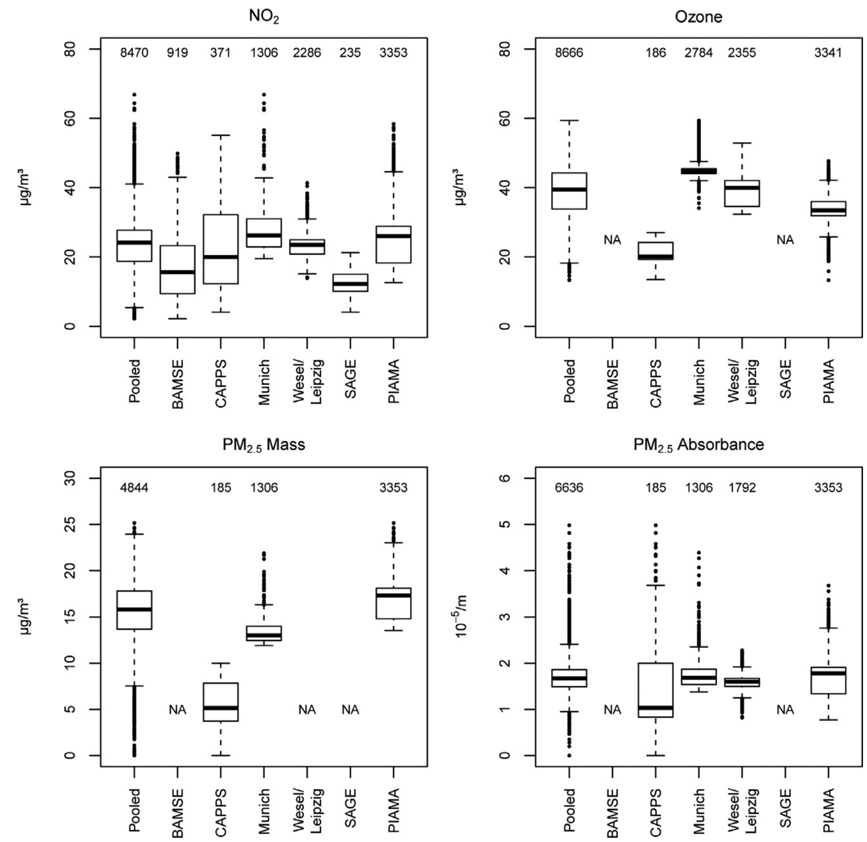

FIG 1. Distribution of air pollutants pooled and by cohort. The interquartile range is indicated by each *box height* and median level by each *dark line*. The number of children with health data that also had available air pollution data are given along the top of each graph. *NA*, Not available.

Our study results also suggest that carriers of the C allele in the rs1927911 SNP in the intron region of the *TLR4* gene may be at an elevated risk of allergic rhinitis. No other studies have documented this association. However, 8 other SNPs in the *TLR*s have been linked to the prevalence of allergic rhinitis, including 1 in the *TLR4* gene (rs4986790).[57] Unfortunately, we did not have data for this SNP in our study. Interestingly, we did not see an association between allergic rhinitis and the rs4696480 SNP in *TLR2*, as has been previously documented in European farmers.[58] Both genetic findings of this study were robust to step-wise exclusion of each cohort.

We found no evidence to support the existence of gene-environment interactions among NO_2, $PM_{2.5}$ mass, $PM_{2.5}$ absorbance, or ozone and 10 SNPs in the *GSTP1*, *TNF*, *TLR2*, and

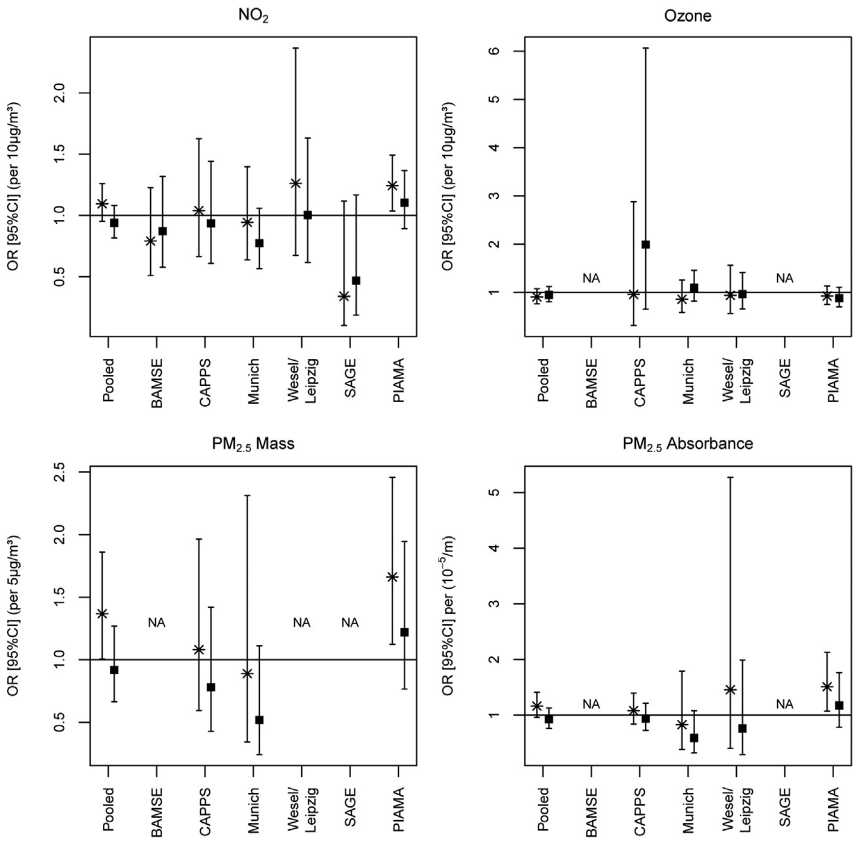

FIG 2. Associations between allergic rhinitis *(stars)* and aeroallergen sensitization *(dark squares)* and NO_2, ozone, $PM_{2.5}$ mass, and $PM_{2.5}$ absorbance. Models were adjusted for city/center, cohort, sex, birth weight, parental history of atopic disease, maternal smoking during pregnancy, exposure to environmental tobacco smoke at the time of follow-up, maternal age at birth, and intervention status (when applicable). *NA*, Not available.

TLR4 genes. We did find a significant risk of sensitization to indoor aeroallergens among minor rs1800629 allele carriers exposed to NO_2; however, this result was not also observed for outdoor aeroallergens. The interaction term between ozone and the rs1695 SNP was also significant for overall aeroallergen sensitization. However, neither the main environmental nor genetic effect estimates were significant for this outcome and SNP.

To date, we are the first to assess the existence of gene-air pollution interactions for allergic rhinitis. However, gene-environment interactions have been reported for other environmental exposures.[59,60] For sensitization, Melén et al[23] reported a significant interaction between NO_x and the rs1695 SNP (*GSTP1*) with the use of the BAMSE cohort. Although we included this cohort in our analysis and examined it individually, we were unable to replicate this finding. However, in the present analysis, sensitization was assessed at 8 years of age and included only aeroallergens, whereas Melén et al[23] examined sensitization to food or aeroallergens at 4 years of age. A recent publication by the BAMSE cohort research group suggests that the adverse effects of air pollution on sensitization may be restricted to gestation and early childhood time points during which the immune system is rapidly developing (allergic rhinitis was not considered).[61] This hypothesis, namely that the adverse effects of TRAP may be limited to early life, may explain why a gene-environment interaction was observed when the BAMSE population was 4 years old but not in the present study in which they are 8. However, we cannot rule out that interaction effects may exist among *GSTP1*, air pollutants, and allergy-related outcomes. An even larger sample size, including a complete cover of variants in *GSTP1,* will likely give further insights into this complex interplay.

Gilliland et al[20,21] also reported positive findings for gene-environment effects for sensitization: nasal IgE levels were increased among genetically susceptible allergic persons after exposure to diesel exhaust particles and secondhand smoke. The discrepancy between these positive findings and our null results may reflect differences in study design, patient populations, and phenotypes studied. Most notably, the studies conducted by Gilliland et al[20,21] involved adult patients and an experimental study design. Furthermore, epigenetic effects were not considered in our study or the other studies but are likely to have important consequences for disease development, as described in a recent update on the current literature on air pollution, genetics (and epigenetics), and allergy.[62]

One of the main issues of studies that examined gene-environment interactions, in addition to many other challenges, is that null findings may simply be due to lack of statistical power.[63] The TAG initiative answers the numerous calls for the need to increase sample sizes by combining cohorts so that we are better poised to fully investigate the relationships and interactions among the genome, the environment, and disease

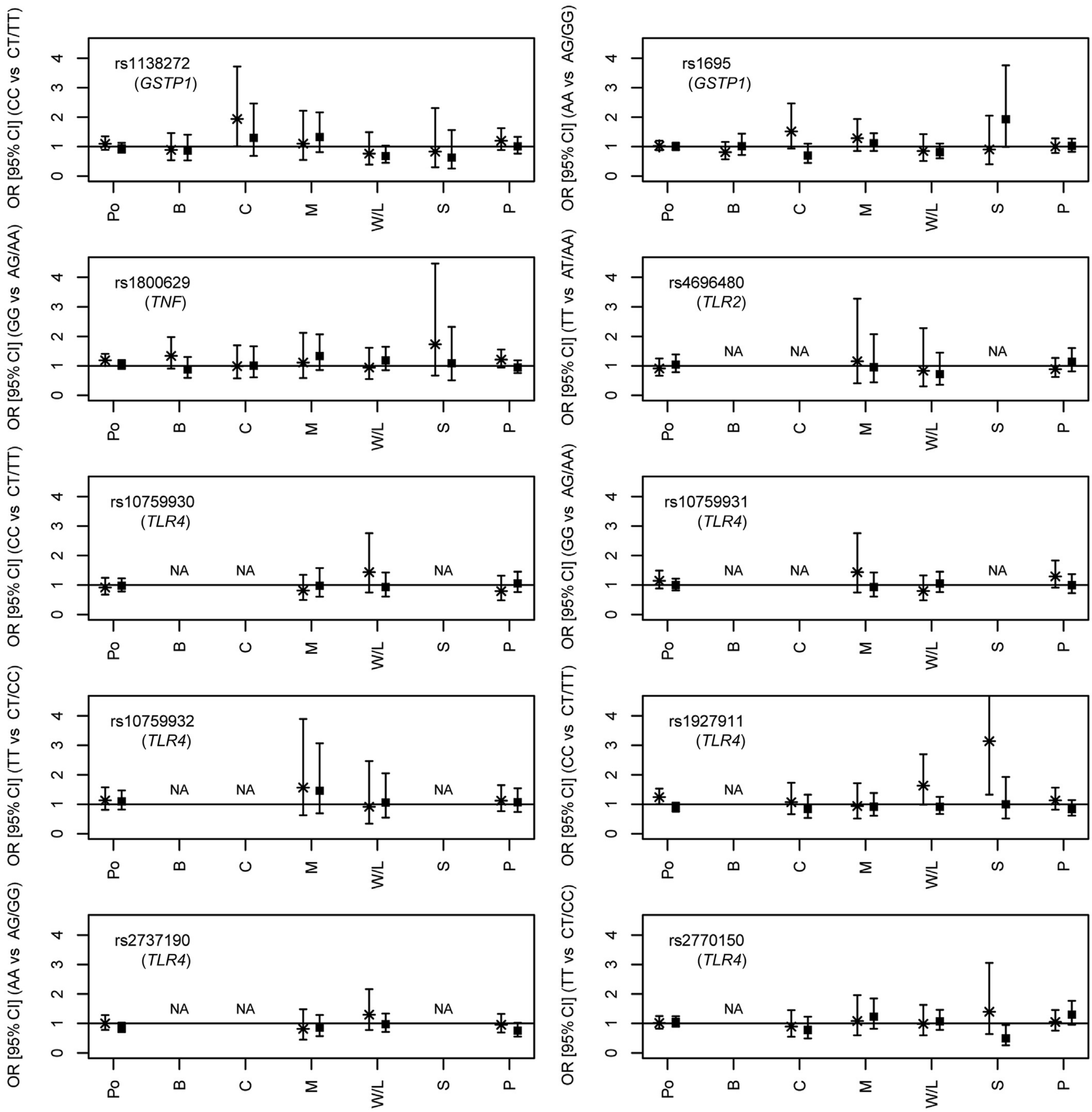

FIG 3. Associations between allergic rhinitis *(stars)* and aeroallergen sensitization *(dark squares)* and single nucleotide polymorphisms. *Po*, Pooled; *B*, BAMSE; *C*, CAPPS; *M*, Munich; *W/L*, Wesel and Leipzig; *S*, SAGE; and *P*, PIAMA. The upper confidence limit in SAGE for rs1927911 and allergic rhinitis is 7.44. Models were adjusted for city/center, cohort, sex, birth weight, parental history of atopic disease, maternal smoking during pregnancy, exposure to environmental tobacco smoke at the time of follow-up, maternal age at birth, and intervention status (when applicable). *NA*, Not available.

development. Nevertheless, we cannot exclude the possibility that our study may still be underpowered to detect real gene-environment interactions. For this reason, we also conducted stratified analyses, for which power may be less likely a concern but can still be limiting. For example, even by combining all available NO_2, health, and covariate data available among minor rs1800629 allele carriers (n = 1360), we were only powered to detect associations with an OR >1.36 (calculated with G*Power version 3.1.3,[64] assuming $\alpha = 0.05$, power = 0.85). Regardless, this limitation is unlikely to hinder the main environmental and genetic effect estimates reported in this study, which have traditionally been estimated

TABLE IV. Associations between air pollution and allergic rhinitis among homozygous major and heterozygous/homozygous minor allele carriers in the pooled data set

	Homozygous major		Heterozygous/ homozygous minor	
SNP	**No.**	**OR (95% CI)**	**No.**	**OR (95% CI)**
NO_2				
rs1138272	3589	0.97 (0.78-1.19)	756	1.20 (0.77-1.87)
rs1695	1902	1.08 (0.82-1.43)	2581	0.96 (0.75-1.21)
rs1800629	2896	0.97 (0.77-1.23)	1351	1.10 (0.78-1.54)
rs4696480	359	1.01 (0.52-1.95)	960	1.24 (0.88-1.73)
rs10759930	561	0.86 (0.48-1.55)	948	1.37 (0.90-2.10)
rs10759931	753	1.27 (0.82-1.95)	1235	1.07 (0.74-1.54)
rs10759932	1004	1.22 (0.88-1.71)	312	0.98 (0.49-1.94)
rs1927911	1400	1.35 (0.99-1.85)	1081	0.77 (0.54-1.11)
rs2737190	936	1.30 (0.86-1.95)	1078	1.05 (0.73-1.53)
rs2770150	1262	0.87 (0.62-1.21)	1208	1.26 (0.90-1.77)
Ozone				
rs1138272	2945	0.92 (0.72-1.18)	639	0.94 (0.51-1.73)
rs1695	1623	0.71 (0.50-1.02)	2266	0.90 (0.68-1.20)
rs1800629	2335	0.81 (0.60-1.09)	1116	0.99 (0.67-1.46)
rs4696480	391	1.54 (0.80-2.94)	1110	0.94 (0.63-1.41)
rs10759930	630	0.95 (0.42-2.15)	1060	0.92 (0.50-1.66)
rs10759931	873	1.21 (0.71-2.04)	1463	1.00 (0.68-1.45)
rs10759932	1141	1.03 (0.69-1.54)	357	1.15 (0.56-2.34)
rs1927911	1422	0.99 (0.67-1.47)	1102	1.10 (0.70-1.72)
rs2737190	1100	1.03 (0.67-1.59)	1262	1.14 (0.75-1.74)
rs2770150	1284	0.99 (0.65-1.50)	1227	1.19 (0.79-1.81)
$PM_{2.5}$ mass				
rs1138272	1903	1.20 (0.76-1.89)	402	1.29 (0.50-3.35)
rs1695	1010	1.72 (0.95-3.13)	1467	0.97 (0.58-1.65)
rs1800629	1514	1.40 (0.87-2.27)	749	0.91 (0.40-2.08)
rs4696480	288	1.52 (0.30-7.58)	721	1.70 (0.81-3.58)
rs10759930	175	0.60 (0.21-1.69)	317	1.41 (0.68-2.95)
rs10759931	419	2.07 (0.74-5.76)	724	1.25 (0.53-2.97)
rs10759932	774	1.74 (0.82-3.69)	231	1.28 (0.26-6.20)
rs1927911	752	1.42 (0.77-2.65)	581	0.94 (0.48-1.82)
rs2737190	550	2.77 (1.07-7.15)*	619	1.07 (0.43-2.66)
rs2770150	676	1.05 (0.57-1.93)	644	1.37 (0.69-2.71)
$PM_{2.5}$ absorbance				
rs1138272	2368	1.02 (0.80-1.31)	505	1.24 (0.70-2.17)
rs1695	1271	1.09 (0.78-1.53)	1828	1.03 (0.77-1.37)
rs1800629	1864	0.99 (0.76-1.30)	932	1.31 (0.85-2.00)
rs4696480	288	1.59 (0.41-6.23)	721	1.56 (0.81-3.00)
rs10759930	390	0.80 (0.52-1.22)	636	1.12 (0.81-1.55)
rs10759931	635	1.62 (0.71-3.72)	1042	1.14 (0.56-2.32)
rs10759932	774	1.66 (0.86-3.18)	231	1.41 (0.35-5.67)
rs1927911	1049	1.14 (0.82-1.60)	818	0.98 (0.71-1.36)
rs2737190	801	1.79 (0.83-3.86)	902	1.11 (0.53-2.33)
rs2770150	948	1.09 (0.82-1.45)	904	0.93 (0.62-1.40)

Models were adjusted for city/center-cohort, sex, birth weight, parental history of atopic disease, maternal smoking during pregnancy, exposure to environmental tobacco smoke at the time of follow-up, maternal age at birth, and intervention status (when applicable).

*Statistically significant result.

with smaller sample sizes. However, we acknowledge the possibility remains that the positive results reported here may be due to chance.

A few limitations should be noted. Common to all studies that combine data sources, the data were not collected with the use of identical strategies across all cohorts. This is an especially relevant concern in this study because differing definitions of allergic rhinitis were used by each cohort, which may have affected the study-specific prevalence estimates. For example, the 2 German cohorts that relied on the report of a doctor diagnosis in the past 12 months had the lowest prevalence rates of allergic rhinitis, although these rates were similar to that reported for Germany in a global study that relied on questionnaire-based report of symptoms.[65] Any misclassification of the disease outcome would likely be nondifferential and would drive the results toward the null. As such, nondifferential misclassification cannot be ruled out as an explanation for our findings. Furthermore, not all participating cohorts were population based, which may influence the prevalence of disease, such as for the CAPPS cohort of children with hereditary allergies. However, our results remained stable when we adjusted for whether a child was a case in the nested case-control cohorts (BAMSE and SAGE), excluded these cases completely from the analysis, or removed each cohort sequentially. Second, the panel of SNPs assessed was selective and may not include other genotypes that could influence the pathogenesis and expression of allergic rhinitis and aeroallergen sensitization. In fact, it is quite likely that a complex interaction of genes is required to determine susceptibility. Our selection was based on the literature that suggests that genes involved in inflammation or oxidative stress metabolism may play a role, and on the availability of the SNP in at least 3 cohorts. Third, although all exposure estimates were individually assigned to each participant, which is a main strength of this study, exposure misclassification remains possible. Furthermore, our approach only considered one air pollutant per analysis. This does not reflect a person's true exposure, which is, in reality, a complex combination of several components. Fourth, we did not have information on the moving patterns of the children from all cohorts. Thus, we were unable to assess the percentage of children for whom an estimation of TRAP exposure at their home address at birth may not reflect exposures in later childhood. A previous examination of this issue found stronger associations between TRAP and allergic diseases for children who had never moved.[9] As such, the effect of moving most likely biased our air pollution results toward the null. Population stratification is also likely of minimal concern because 95.1% of our study participants were of European descent. Finally, selective dropout is unlikely to have affected the main genetic results of this study because it is improbable that a person's genotype influenced his or her decision to participate.

In conclusion, a pooled analysis of 6 birth cohorts suggests that the generally null effect of TRAP on allergic rhinitis and sensitization is not modified by 10 SNPs in the *GSTP1, TNF, TLR2,* and *TLR4* genes. Although TRAP increased the risk of allergic rhinitis in the pooled analysis, this result was not robust to single cohort exclusions. Children with at least 1 minor rs1800629 allele in their *TNF* gene or 1 minor rs1927911 allele in their *TLR4* gene may be at a higher risk of allergic rhinitis by school age. This finding has important public health relevance because both SNPs are present in a large proportion of the population (31.2% and 43.5%, respectively, in this study). The biological mechanisms behind these possible associations remain unknown.

We thank all children and parents for their cooperation, as well as all technical and administrative support staff and the medical and field work teams. We also thank Dr Kees De Hoogh at Imperial College for providing the

ozone estimates derived from the APMoSPHERE project. Some of the results of this study have been previously published in the form of an abstract.[66]

Key messages

- **A pooled analysis of 6 birth cohorts suggests that the generally null effect of traffic-related air pollution on allergic rhinitis and sensitization is not modified by 10 tested single nucleotide polymorphisms in the *GSTP1, TNF, TLR2,* and *TLR4* genes.**
- **Traffic-related air pollution did not consistently increase the risk of allergic rhinitis onset in a pooled analysis of 6 birth cohorts.**
- **Children with at least 1 minor rs1800629 allele in the *TNF* gene or 1 minor rs1927911 allele in the *TLR4* gene may be at a higher risk of developing allergic rhinitis by school age.**

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TABLE E1. Pooled and cohort specific associations between allergic rhinitis and aeroallergen sensitization and air pollutants

	BAMSE		CAPPS		Munich		Wesel/Leipzig		SAGE		PIAMA		Pooled	
	No.	**OR (95% CI)**	**No.**	**OR (95% CI)**	**No.**	**OR (95% CI)**	**No.**	**OR (95% CI)**	**No.**	**OR (95% CI)**	**No.**	**OR (95% CI)**	**No.**	**OR (95% CI)**
Allergic rhinitis														
NO_2	897	0.79 (0.51-1.23)	368	1.04 (0.67-1.63)	1028	0.94 (0.64-1.40)	1740	1.26 (0.67-2.37)	171	0.34 (0.10-1.12)	3160	1.24 (1.04-1.49)*	7364	1.10 (0.95-1.26)
Ozone	—	—	186	0.96 (0.32-2.88)	2163	0.86 (0.59-1.26)	1793	0.94 (0.57-1.56)		—	3151	0.93 (0.75-1.13)	7293	0.91 0.77-1.08)
$PM_{2.5}$ mass	—	—	185	1.08 (0.59-1.96)	1028	0.89 (0.34-2.31)	—	—	—	—	3160	1.66 (1.12-2.46)*	4373	1.37 (1.01-1.86)*
$PM_{2.5}$ absorbance	—	—	185	1.08 (0.84-1.40)	1028	0.83 (0.38-1.79)	1339	1.46 (0.40-5.27)	—	—	3160	1.51 (1.07-2.13)*	5712	1.16 (0.96-1.41)
Aeroallergen sensitization														
NO_2	751	0.87 (0.58-1.32)	355	0.94 (0.61-1.44)	617	0.77 (0.57-1.06)	940	1.00 (0.62-1.63)	215	0.47 (0.19-1.17)	1725	1.10 (0.89-1.37)	4603	0.94 (0.82-1.08)
Ozone	—	—	177	1.99 (0.66-6.06)	1276	1.10 (0.82-1.46)	961	0.97 (0.66-1.41)	—	—	1718	0.88 (0.70-1.11)	4132	0.95 (0.81-1.12)
$PM_{2.5}$ mass	—	—	176	0.78 (0.43-1.42)	617	0.52 (0.24-1.11)	—	—	—	—	1725	1.22 (0.77-1.94)	2518	0.92 (0.66-1.27)
$PM_{2.5}$ absorbance	—	—	176	0.94 (0.72-1.21)	617	0.59 (0.32-1.08)	699	0.76 (0.29-1.99)	—	—	1725	1.17 (0.78-1.76)	3217	0.93 (0.76-1.13)

Models were adjusted for city/center, cohort, sex, birth weight, parental history of atopic disease, maternal smoking during pregnancy, exposure to environmental tobacco smoke at the time of follow-up, maternal age at birth, and intervention status (when applicable).

*Statistically significant result.

TABLE E2. Pooled and cohort-specific associations between allergic rhinitis and aeroallergen sensitization, and each SNP

	BAMSE		CAPPS		Munich		Wesel/Leipzig		SAGE		PIAMA		Pooled	
	No.	OR (95% CI)	No.	OR (95% CI)	No.	OR (95% CI)	No.	OR (95% CI)	No.	OR (95% CI)	No.	OR (95% CI)	No.	OR (95% CI)
Allergic rhinitis														
rs1138272	841	0.89 (0.54-1.46)	344	1.94 (1.01-3.72)*	716	1.10 (0.55-2.21)	913	0.76 (0.39-1.49)	134	0.83 (0.30-2.31)	1791	1.20 (0.88-1.63)	4739	1.09 (0.89-1.35)
rs1695	876	0.81 (0.57-1.16)	344	1.51 (0.93-2.46)	1107	1.28 (0.85-1.93)	843	0.85 (0.51-1.42)	131	0.90 (0.40-2.05)	1776	1.00 (0.79-1.28)	5077	1.02 (0.87-1.20)
rs1800629	833	1.34 (0.90-1.98)	345	0.99 (0.58-1.70)	655	1.11 (0.58-2.12)	860	0.94 (0.55-1.61)	135	1.73 (0.67-4.47)	1773	1.21 (0.95-1.55)	4601	1.19 (1.00-1.41)*
rs4696480	—	—	—	—	349	1.16 (0.41-3.28)	313	0.83 (0.30-2.28)	—	—	843	0.89 (0.62-1.27)	1505	0.91 (0.66-1.25)
rs10759930	—	—	346	0.82 (0.49-1.35)	655	1.44 (0.75-2.76)	861	0.79 (0.48-1.32)	—	—	—	—	1862	0.92 (0.68-1.25)
rs10759931	—	—	—	—	655	1.44 (0.75-2.76)	861	0.80 (0.48-1.33)	—	—	824	1.29 (0.91-1.83)	2340	1.15 (0.88-1.49)
rs10759932	—	—	—	—	346	1.56 (0.63-3.89)	314	0.91 (0.34-2.46)	—	—	841	1.12 (0.76-1.65)	1501	1.13 (0.81-1.57)
rs1927911	—	—	345	1.07 (0.66-1.73)	655	0.94 (0.51-1.71)	858	1.63 (0.99-2.69)	135	3.14 (1.33-7.44)*	841	1.13 (0.81-1.56)	2834	1.24 (1.01-1.53)*
rs2737190	—	—	—	—	654	0.82 (0.45-1.48)	861	1.30 (0.78-2.16)	—	—	851	0.96 (0.69-1.32)	2366	1.00 (0.78-1.28)
rs2770150	—	—	345	0.89 (0.55-1.45)	654	1.08 (0.60-1.96)	859	0.99 (0.60-1.63)	136	1.40 (0.64-3.06)	828	1.05 (0.76-1.46)	2822	1.02 (0.82-1.25)
Aeroallergen sensitization														
rs1138272	706	0.87 (0.53-1.40)	332	1.30 (0.68-2.46)	546	1.33 (0.81-2.16)	842	0.68 (0.45-1.03)	169	0.63 (0.25-1.56)	1496	1.01 (0.76-1.33)	4091	0.95 (0.79-1.13)
rs1695	735	1.02 (0.72-1.44)	333	0.70 (0.44-1.10)	1129	1.11 (0.86-1.45)	866	0.82 (0.60-1.11)	167	1.93 (0.99-3.76)	1486	1.02 (0.82-1.27)	4716	1.00 (0.88-1.13)
rs1800629	699	0.88 (0.59-1.30)	333	1.01 (0.61-1.66)	507	1.33 (0.86-2.07)	795	1.18 (0.85-1.65)	171	1.09 (0.51-2.32)	1481	0.95 (0.75-1.19)	3986	1.04 (0.90-1.20)
rs4696480	—	—	—	—	193	0.95 (0.44-2.07)	231	0.72 (0.36-1.45)	—	—	721	1.14 (0.81-1.60)	1145	1.04 (0.79-1.39)
rs10759930	—	—	334	0.98 (0.61-1.57)	507	0.93 (0.61-1.43)	796	1.05 (0.77-1.45)	—	—	—	—	1637	0.98 (0.79-1.23)
rs10759931	—	—	—	—	507	0.93 (0.61-1.43)	796	1.05 (0.76-1.45)	—	—	707	1.01 (0.73-1.37)	2010	1.00 (0.82-1.22)
rs10759932	—	—	—	—	190	1.46 (0.69-3.07)	232	1.06 (0.55-2.05)	—	—	719	1.07 (0.74-1.54)	1141	1.10 (0.82-1.47)
rs1927911	—	—	333	0.84 (0.54-1.32)	507	0.92 (0.61-1.38)	794	0.91 (0.67-1.25)	171	1.00 (0.52-1.92)	720	0.84 (0.61-1.14)	2525	0.89 (0.75-1.06)
rs2737190	—	—	—	—	506	0.86 (0.57-1.29)	796	0.98 (0.71-1.33)	—	—	727	0.76 (0.56-1.02)	2029	0.85 (0.70-1.03)
rs2770150	—	—	333	0.78 (0.49-1.23)	507	1.23 (0.82-1.85)	794	1.07 (0.78-1.46)	172	0.49 (0.26-0.95)	709	1.30 (0.96-1.77)	2515	1.05 (0.89-1.25)

Models were adjusted for city/center, cohort, sex, birth weight, parental history of atopic disease, maternal smoking during pregnancy, exposure to environmental tobacco smoke at the time of follow-up, maternal age at birth, and intervention status (when applicable).

*Statistically significant result.