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Atopic dermatitis: Interaction between genetic variants of *GSTP1*, *TNF*, *TLR2*, and *TLR4* and air pollution in early life

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Abstract

Background: Associations between traffic-related air pollution (TRAP) and childhood atopic dermatitis (AD) remain inconsistent, possibly due to unexplored gene-environment interactions. The aim of this study was to examine whether a potential effect of TRAP on AD prevalence in children is modified by selected single nucleotide polymorphisms (SNPs) related to oxidative stress and inflammation.

Methods: Doctor-diagnosed AD up to age 2 years and at 7-8 years, as well as AD symptoms up to age 2 years, was assessed using parental-reported questionnaires in six birth cohorts (N = 5685). Associations of nitrogen dioxide (NO_2) estimated at the home address of each child at birth and nine SNPs within the *GSTP1*, *TNF*, *TLR2*, or *TLR4* genes with AD were examined. Weighted genetic risk scores (GRS) were calculated from the above SNPs and used to estimate combined marginal genetic effects of oxidative stress and inflammation on AD and its interaction with TRAP.

Results: GRS was associated with childhood AD and modified the association between NO_2 and doctor-diagnosed AD up to the age of 2 years (*P*(interaction) = .029). This interaction was mainly driven by a higher susceptibility to air pollution in *TNF* rs1800629 minor allele (A) carriers. TRAP was not associated with the prevalence of AD in the general population.

Conclusions: The marginal genetic association of a weighted GRS from *GSTP1*, *TNF*, *TLR2*, and *TLR4* SNPs and its interaction with air pollution supports the role of oxidative stress and inflammation in AD.

KEYWORDS
atopic eczema, gene-environment interaction, weighted genetic risk scores

1 | INTRODUCTION

Many studies have investigated the role of traffic-related air pollution (TRAP) in childhood allergic diseases. In particular, recent evidence suggests that exposure to TRAP in early life contributes to the development of asthma throughout childhood and adolescence.[1,2] Atopic dermatitis (AD) is the most common inflammatory skin disorder in childhood[3] and is considered a beginning of the atopic march,[4] progressing to subsequent allergic diseases such as allergic rhinitis and asthma. Few studies have examined whether TRAP is associated with AD[2] and the results are inconsistent. There is some evidence supporting adverse effects of TRAP on AD and current itchy rashes.[5-7] However, several studies do also report null effects[8-10] and additional research is warranted to fully understand the role of TRAP in the development of AD.

As gene-environment (G × E) interactions have been found to play an important role in the association between air pollution and allergy-related diseases,[11] the inconsistencies found in previous studies of TRAP and AD may be due to genetic variation. Specifically, genes belonging to the glutathione S-transferase (GST) family are of particular interest because of their role in cellular protection against oxidative stress, which is a potential pathway for toxic air pollution effects.[12] Recently, evidence has found that children with GST pi 1 (*GSTP1)* and GST Mu 1 (*GSTM1)* genotypes may constitute a susceptible population at increased risk of asthma associated with TRAP[13,14] and of childhood AD associated with prenatal smoke exposure.[15]

In addition, there is growing evidence that air pollutants activate Toll-like receptor (TLR) signaling, resulting in a pro-inflammatory response in the lung,[16] and a previous study in the PIAMA cohort identified a gene by TRAP interaction for *TLR2* and *TLR4* variants with respect to asthma.[17] Furthermore, there is some evidence that *TNF* polymorphisms modify the association between TRAP and allergic sensitization.[18]

In addition, studies indicate an association of GSTs,[19] *TLR4* and *TLR2*,[20] and *TNF* variants[21] with AD.

We used a harmonized dataset from six birth cohort studies in Canada and Europe to determine the effect of residential TRAP exposure on AD up to the age of 2 years and at the age of 7-8 years and whether this association was modified by variants in genes related to oxidative stress and inflammation.

2 | METHODS

2.1 | Study collective

This study was part of the international Traffic, Asthma, and Genetics (TAG) study, which investigated whether the effects of TRAP

exposure on childhood asthma, hay fever, and AD were modified by specific candidate genes related to inflammation and oxidative stress.[13,22,23] The TAG study was a collaboration of four European (BAMSE, GINIplus, LISAplus, and PIAMA) and two Canadian (CAPPS and SAGE) birth cohort studies.

Ethics approval was obtained from the local authorized institutional review boards. A detailed description of the methodology used to harmonize the data and to create a pooled, central dataset is described elsewhere.[23] In total, 15 299 children were included in the central TAG database.

2.2 | AD phenotypes

Data on childhood AD were obtained using parental-reported questionnaire data on doctor-diagnosed AD and AD symptoms. We created three harmonized outcome variables that were available in at least four cohorts: AD symptoms up to the age of 2 years, doctor-diagnosed AD up to the age of 2 years, and doctor-diagnosed AD at the age of 7-8 years (see supplement for more details).

2.3 | Air pollution exposure

Annual average concentrations of NO_2 were modeled for children's birth home addresses using land-use regression models, except for the BAMSE cohort, which used dispersion modeling based on wind speed, direction, and precipitation.[13]

Exposures at the time of birth were assigned to geocoded birth addresses. Similar to previous TAG studies,[13,22] NO_2 was used as the main surrogate for TRAP exposure in all analyses.

2.4 | Genotyping

We investigated variants related to oxidative stress (*GSTP1*) and inflammation (*TNF*, *TLR2*, and *TLR4*). Details on the genotyping procedures are summarized in the supplementary material. All SNPs had a genotyping success rate >93% and did not violate the Hardy-Weinberg Equilibrium.

Candidate genes were selected based on their involvement in the different biological pathways under consideration and on their availability in the central TAG database for at least three cohorts. The single nucleotide polymorphisms (SNPs) used in this analysis are listed in Table S2.

2.5 | Covariates

Data on covariates were obtained by questionnaire. Covariates in the adjusted models were selected a priori based upon findings from previous studies[13,22] and included the following: study region/center, cohort (only in the pooled data analysis), sex, parental history of allergy (excluded for models investigating the marginal effect of SNPs due to the potential intermediate effect), maternal smoking during pregnancy, any SHS up to the age of 2 years at the child's home, and maternal age at childbirth. Furthermore, we considered participation in the intervention groups as an additional covariate for GINIplus, CAPPS, and PIAMA and case-control status in SAGE (asthma at the age of 7 years) and BAMSE (wheeze at the age of 4 years).

2.6 | Statistical analysis

2.6.1 | Traffic-related air pollution and AD

Multiple logistic regression models were used to analyze the association between TRAP exposure and AD (effect of TRAP) for each cohort separately as well as in a pooled analysis. Air pollution (NO_2) was included as continuous variables with an increment of 10 $\mu g/m^3$. This analysis workflow followed previous TAG publications on asthma[13] and allergic rhinitis.[22]

2.6.2 | Construction of weighted genetic risk scores

To estimate the role of the oxidative stress and inflammation on AD in general and on air pollution-induced AD in particular, we calculated, for each cohort separately, weighted genetic risk scores (GRS). GRS aggregate measured genetic effects and therefore increase the power to detect gene-environment interactions.[24] GRS further serve as a simple statistical approach for the complex biological pathways through which air pollution could influence AD.

Weighted GRS were defined as a weighted sum of the number of risk alleles of the considered SNPs. The weights were gained from the β-estimates (=ln(OR)) of the marginal genetic effect (associations between each single SNP and AD) estimated in the pooled single SNP analysis for each phenotype separately. The signs of the marginal genetic effect estimates were used for the definition of risk alleles in the GRS: If the β-estimates were >0, the minor allele was defined as the risk allele, whereas if the β-estimates were <0, the major allele was defined as the risk allele. The cohort-specific GRS were based on all SNPs that were available in at least 50% of the cohort, leading to a different number of SNPs considered in the GRS in each cohort (see Tables S8-S10). More details on the construction of weighted GRS are given in the supplementary material.

2.6.3 | Marginal genetic and interaction effects

We estimated in each cohort separately the marginal genetic effect of the dichotomized GRS on AD, as well as the interaction of dichotomized GRS with the continuous air pollution exposure (called GRS × E interaction).

In a next step, a fixed-effect meta-analysis of all cohort-specific marginal GRS and GRS-environment interaction (GRS × E) effect estimates was performed to provide overall estimates and 95% confidence intervals. The *Q* test was used to test for heterogeneity between the cohorts.

For a better interpretation of the GRS × E findings, we assessed effect modifications of the association between TRAP and AD by each single SNP (dominant model).

In a sensitivity analysis, we calculated cohort-specific GRS from SNPs that were available in all cohorts (*GSTP1* and *TNF* variants).

TABLE 1 Study characteristics of the pooled data

	Pooled	BAMSE[b]	CAPPS[b]	GINIplus/LISAplus[b] (Munich)	GINIplus/LISAplus[b] (Wesel & Leipzig)	SAGE[b]	PIAMA[b]
N (%)	5685[a]	979 (17.22%)	348 (6.12%)	826 (14.53%)	1330 (23.39%)	184 (3.24%)	2018 (35.50%)
Males, n/N (%)	2978/5685 (52.38%)	521/979 (53.22%)	189/348 (54.31%)	437/826 (52.91%)	692/1330 (52.03%)	101/184 (54.89%)	1038/2018 (51.44%)
Parental history of allergies, n/N (%)	3106/5682 (54.66%)	562/979 (57.41%)	321/348 (92.24%)	464/824 (56.31%)	491/1329 (36.95%)	130/184 (70.65%)	1138/2018 (56.39%)
Intervention participation, n/N (%)	1295/5685 (22.78%)	n.a.	183/348 (52.59%)	267/826 (32.32%)	360/1330 (27.07%)	n.a.	485/2018 (24.03%)
Cases[c], n/N (%)	415/5683 (7.30%)	341/979 (34.83%)	n.a.	n.a.	n.a.	74/182 (40.66%)	n.a.
Second-hand smoke during pregnancy, n/N (%)	792/5408 (14.64%)	138/979 (14.10%)	29/346 (8.38%)	97/730 (13.29%)	188/1174 (16.01%)	21/181 (11.60%)	319/1998 (15.97%)
Any second-hand smoke up to the age of 2, n/N (%)	1844/5452 (33.82%)	220/975 (22.56%)	82/348 (23.56%)	209/817 (25.58%)	500/1305 (38.31%)	n.a.	833/2007 (41.50%)
Any second-hand smoke up to the age of 8, n/N (%)	2225/5682 (39.16%)	268/979 (27.37%)	96/348 (27.59%)	276/826 (33.41%)	639/1330 (48.05%)	36/181 (19.89%)	910/2018 (45.09%)
Maternal age at birth (y), mean (SD)	30.91 (4.13)	30.69 (4.55)	31.83 (5.03)	32.33 (4.06)	30.36 (3.79)	30.25 (4.72)	30.68 (3.76)
Symptoms of AD up to 2 y, n/N (%)	1146/5076 (22.58%)	270/979 (27.58%)	n.a.	138/813 (16.97%)	230/1293 (17.79%)	n.a.	508/1991 (25.51%)
Doctor-diagnosed AD up to 2 y, n/N (%)	1090/5410 (20.15%)	203/979 (20.74%)	40/342 (11.70%)	128/818 (15.65%)	230/1291 (17.82%)	n.a.	489/1980 (24.70%)
Doctor-diagnosed AD at 7 or 8 y, n/N (%)	440/5132 (8.57%)	96/884 (10.86%)	45/348 (12.93%)	27/725 (3.72%)	44/1121 (3.93%)	23/184 (12.50%)	205/1870 (10.96%)

n.a., not available in this cohort; SD, standard deviation; y, years; AD, atopic dermatitis.

[a]Children with data on atopic dermatitis (AD), air pollution exposure, and genotyped data for at least one single nucleotide polymorphism.

[b]See supplement for full names of cohorts.

[c]SAGE: asthma at the age of 7, BAMSE: wheeze at the age of 4.

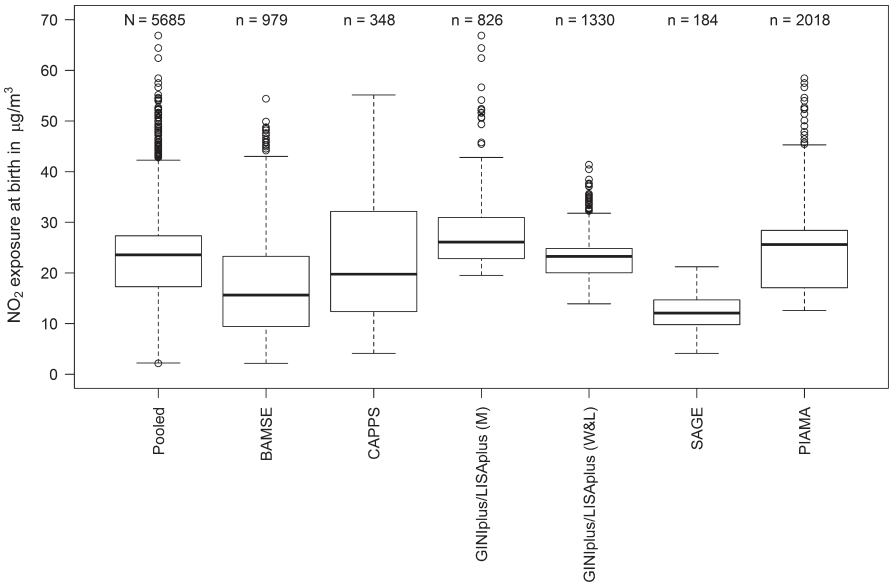

FIGURE 1 Box plots of estimated NO_2 concentrations at the participants' birth addresses in the pooled data and in each study separately. M, Munich; W&L, Wesel & Leipzig

TABLE 2 Association between NO_2 exposure at birth and atopic dermatitis (AD) up to the age of 2 and at 7 or 8 y

	Reported symptoms up to the age of 2 y			Doctor-diagnosed up to the age of 2 y			Doctor-diagnosed at the age of 7 or 8 y		
	OR (95% CI)	*P*-value	n	OR (95% CI)	*P*-value	n	OR (95% CI)	*P*-value	n
Pooled	1.02 (0.88-1.19)	.782	4806	0.95 (0.82-1.11)	.524	5135	1.00 (0.80-1.24)	.993	4879
BAMSE	1.03 (0.76-1.40)	.848	976	1.06 (0.76-1.48)	.752	976	0.77 (0.48-1.25)	.296	881
CAPPS	n.a.	n.a.	n.a.	0.56 (0.28-1.11)	.096	340	1.08 (0.54-2.17)	.828	346
GINIplus/LISAplus (Munich)	0.98 (0.71-1.35)	.891	716	0.98 (0.71-1.36)	.914	720	1.26 (0.75-2.11)	.378	637
GINIplus/LISAplus (Wesel & Leipzig)	0.68 (0.41-1.12)	.132	1148	0.77 (0.47-1.26)	.297	1145	0.74 (0.26-2.11)	.573	992
SAGE	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	1.52 (0.31-7.52)	.606	170
PIAMA	1.10 (0.88-1.37)	.427	1964	0.97 (0.77-1.22)	.777	1952	1.01 (0.73-1.38)	.968	1849

All models were adjusted for city/center, cohort (only in the pooled dataset), sex, parental history of allergy, maternal smoking during pregnancy, current exposure to second-hand smoke up to the age of 8, and maternal age at childbirth. Furthermore, we considered the participation in the intervention groups as an additional covariate for GINIplus, CAPPS, and PIAMA and case-control status in SAGE (asthma at the age of 7) and BAMSE (wheeze at the age of 4). n.a., not available in this cohort.
Odds ratios (OR) and 95% confidence intervals (CI) are given per increase of 10 $\mu g/m^3$ in NO_2.

Effect estimates were calculated for crude and adjusted models and are presented as odds ratios (OR) with 95% confidence intervals (95% CI).

3 | RESULTS

3.1 | Characterization of the study population

In total, 5685 children had data on AD, air pollution exposure, and genotype data for at least one SNP (Tables 1 and S2).

In the pooled dataset, 22.6% of the children had symptoms of AD by 2 years, 20.2% had doctor-diagnosed AD by 2 years, and 8.6% had doctor-diagnosed AD at the ages of 7 or 8 years (Table 1).

Nitrogen dioxide (NO_2) distributions for Germany (GINIplus and LISAplus) and the Netherlands (PIAMA) were similar, while those for Canada (SAGE and CAPPS) and Sweden (BAMSE) indicated slightly lower mean concentrations (Figure 1).

Table S4 reports genotype frequencies for the pooled data and Table S5 the linkage disequilibrium (LD) between the analyzed SNPs. Only the two *TLR2* SNPs were in moderate LD (r^2 = .54).

3.2 | Traffic-related air pollution and AD

There was no association between NO_2 exposure and AD, neither in the pooled dataset nor in the cohort-specific data (Table 2). Results did not differ significantly when considering second-hand smoke exposure up to the age of 2 years or parental education as additional confounders (Tables S6 and S7).

TABLE 3 Association between oxidative stress single nucleotide polymorphisms (additive model) and atopic dermatitis (AD) in the pooled dataset

	Reported symptoms up to the age of 2 y			Doctor-diagnosed up to the age of 2 y			Doctor-diagnosed at the age of 7 or 8 y		
	OR (95% CI)	*P*-value	n	OR (95% CI)	*P*-value	n	OR (95% CI)	*P*-value	n
GSTP1 rs1138272	0.71 (0.49-1.02)	.066	4255	1.05 (0.73-1.49)	.806	4582	0.72 (0.41-1.24)	.229	4387
GSTP1 rs1695	1.04 (0.84-1.29)	.706	4429	1.09 (0.88-1.34)	.442	4753	0.96 (0.70-1.31)	.790	4514
TNF rs1800629	1.03 (0.79-1.36)	.823	4146	**0.72 (0.54-0.96)**	**.025**	**4474**	**0.65 (0.43-0.99)**	**.044**	**4289**
TLR4 rs2770150	1.00 (0.73-1.37)	.992	2252	1.15 (0.85-1.57)	.357	2585	0.83 (0.52-1.32)	.426	2517
TLR4 rs10759931	0.97 (0.73-1.29)	.819	2247	0.81 (0.60-1.08)	.155	2243	0.80 (0.50-1.28)	.355	2002
TLR4 rs10759932	0.94 (0.56-1.59)	.816	1448	0.85 (0.50-1.46)	.566	1440	0.82 (0.36-1.89)	.643	1317
TLR4 rs1927911	0.97 (0.69-1.34)	.835	2263	1.17 (0.85-1.60)	.347	2596	**1.57 (1.00-2.47)**	**.049**	**2529**
TLR2 rs4696480	0.91 (0.65-1.28)	.598	1452	1.05 (0.75-1.48)	.783	1442	1.04 (0.62-1.75)	.886	1320
TLR2 rs1898830	1.07 (0.69-1.64)	.771	893	1.10 (0.74-1.65)	.635	1224	1.01 (0.62-1.65)	.956	1336

Bold: *P*-values at nominal significance ($P < .05$); None of the *P*-values passed the Bonferroni threshold ($\alpha = 0.05/9 \approx 0.005$). All models were adjusted for city/center, cohort, sex, maternal smoking during pregnancy, current exposure to second-hand smoke up to the age of 8, and maternal age at childbirth. Furthermore, we considered the participation in the intervention groups as additional covariate for GINIplus, CAPPS, and PIAMA.

3.3 | Marginal genetic effects

The marginal genetic effects of all considered SNPs that were used as weights for the weighted GRS are summarized in Table 3. None of the single SNPs passed the Bonferroni threshold.

We found a significant association between the GRS from *GSTP1*, *TNF*, *TLR2*, and *TLR4* SNPs and doctor-diagnosed AD up to the age of 2 years and at the age of 7-8 years (meta-analyzed odds ratios [95% confidence intervals] 1.22 [1.04-1.44] and 1.34 [1.06-1.69], respectively) (Figure 2B-i and C-i). The associations were similar for the GRS that were only based on *GSTP1* and *TNF* SNPs (Figure S1B-i and C-i), which were available for all cohorts. There was no evidence of heterogeneity referring to the *Q* test.

3.4 | Gene-environment interactions

The GRS modified the association between NO_2 exposure and doctor-diagnosed AD up to the age of 2 years (*P*(interaction) = .029 for the general GRS [Figure 2B-ii] and *P*(interaction) = .008 for the GRS from *GSTP1* and *TNF* SNPs only [Figure S1B-ii]). However, associations between NO_2 and AD were neither significant in subjects with a low GRS nor in subjects with a high GRS (Figure 3B-i).

The interaction effect was mainly driven by *TNF* rs1800629 that achieved the highest weight for the calculation of the GRS (Table S8). In this regard, minor allele (A) carriers were more susceptible to air pollution-induced doctor-diagnosed AD up to the age of 2 years.

4 | DISCUSSION

This study is the largest consortium to examine the association between TRAP and AD in up to 5685 children, and the largest to examine the interaction between TRAP and four candidate genes of oxidative stress and inflammation (*GSTP1*, *TLR2*, *TLR4*, and *TNF*) on AD. Combining all considered SNPs in a weighted GRS, our results show that genetic susceptibility to oxidative stress and inflammation was marginally associated with the prevalence of childhood AD (meta-analyzed odds ratios [95% confidence intervals] for doctor-diagnosed AD up to the age of 2 years: 1.22 [1.04-1.44] [$P = .016$]) and modified risk of air pollution-induced AD (meta-analyzed *P*-value for interaction term: $P = .029$).

4.1 | Traffic-related air pollution and AD

In our pooled analysis of six birth cohorts, TRAP was not associated with the prevalence of childhood AD. We did not find an association in pooled or in any of the cohort-specific analyses. Our findings are in line with findings of other studies from Western countries showing null effects for associations between early life exposure to NO_2 and childhood AD, for example, in a Spanish birth cohort of 2199 infants[8] or in a cross-sectional study of 4901 children from France.[10] In contrast, other studies showed associations between soot and doctor-diagnosed eczema at 6 years in 2578 children of the German GINIplus/LISAplus study,[6] between self-reported truck traffic on the street of residence and eczema symptoms in 315 572 children of the International Study of Asthma and Allergies in Childhood (ISAAC),[25] and between mean annual NO_2 levels and AD in 91 642 children of the National Survey of Children's Health.[26] In this regard, the association between air pollution and AD remains inconsistent and further research is needed to investigate the impact of air pollution on AD, for example, with more detailed AD phenotypes incorporating severity of AD symptoms or allergic sensitization.

4.2 | Marginal genetic and gene-environment interaction effects

We found some indication for an impact of oxidative stress and inflammation SNPs on AD, identified using a combined analysis in which all considered SNPs were incorporated in a weighted GRS.

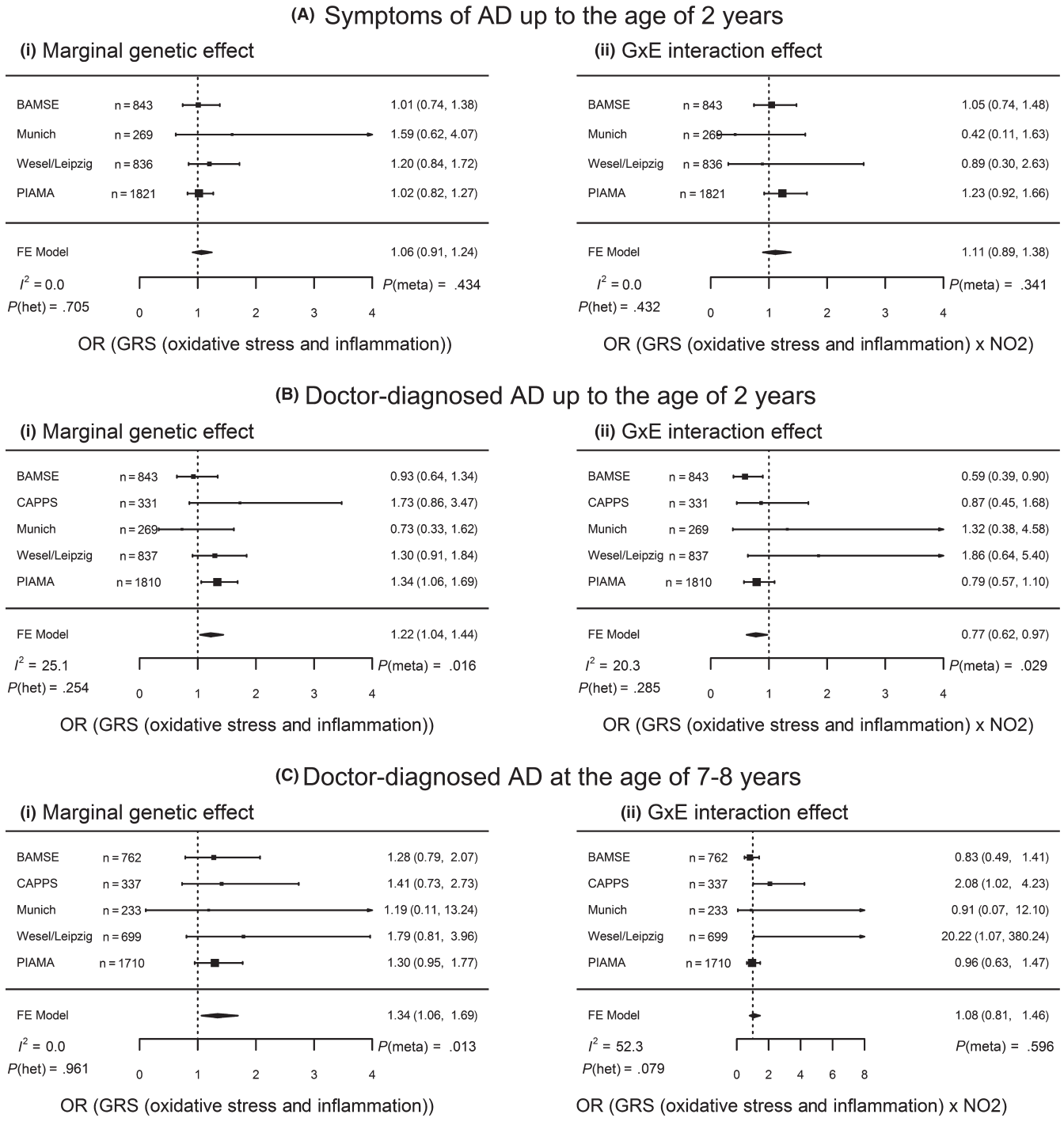

FIGURE 2 Association between the weighted genetic risk score (GRS) for oxidative stress and inflammation single nucleotide polymorphisms (SNPs) (weights from pooled single SNPs analysis) and atopic dermatitis (AD) (i) and GRS × E interaction with NO_2 exposure at birth on AD (ii). All models were adjusted for city/center, cohort, sex, parental history of allergy, maternal smoking during pregnancy, exposure to second-hand smoke (SHS) up to the age of 2, and maternal age at childbirth. Furthermore, we considered the participation in the intervention groups as additional covariate for GINIplus, CAPPS, and PIAMA and case-control status in SAGE (asthma at the age of 7) and BAMSE (wheeze at the age of 4). OR and 95% confidence intervals are given for each cohort separately and combined by fixed-effect (FE) meta-analysis (including *P*-value (*P*(meta))). I^2 is a measure of heterogeneity between cohorts, and *P*(het) is a *P*-value for the *Q* test of heterogeneity. Munich: GINIplus/LISAplus Munich; Wesel/Leipzig: GINIplus/LISAplus Wesel/Leipzig

Furthermore, a statistically significant interaction between the GRS and TRAP on the prevalence of childhood AD up to the age of 2 years was found. This interaction was mainly driven by a higher susceptibility to air pollution-induced AD in *TNF* rs1800629 minor allele (A) carriers. This is in line with Melén et al who showed that the effect of TRAP on childhood allergy appears to be modified by *TNF* (and *GSTP1*) variants.[18]

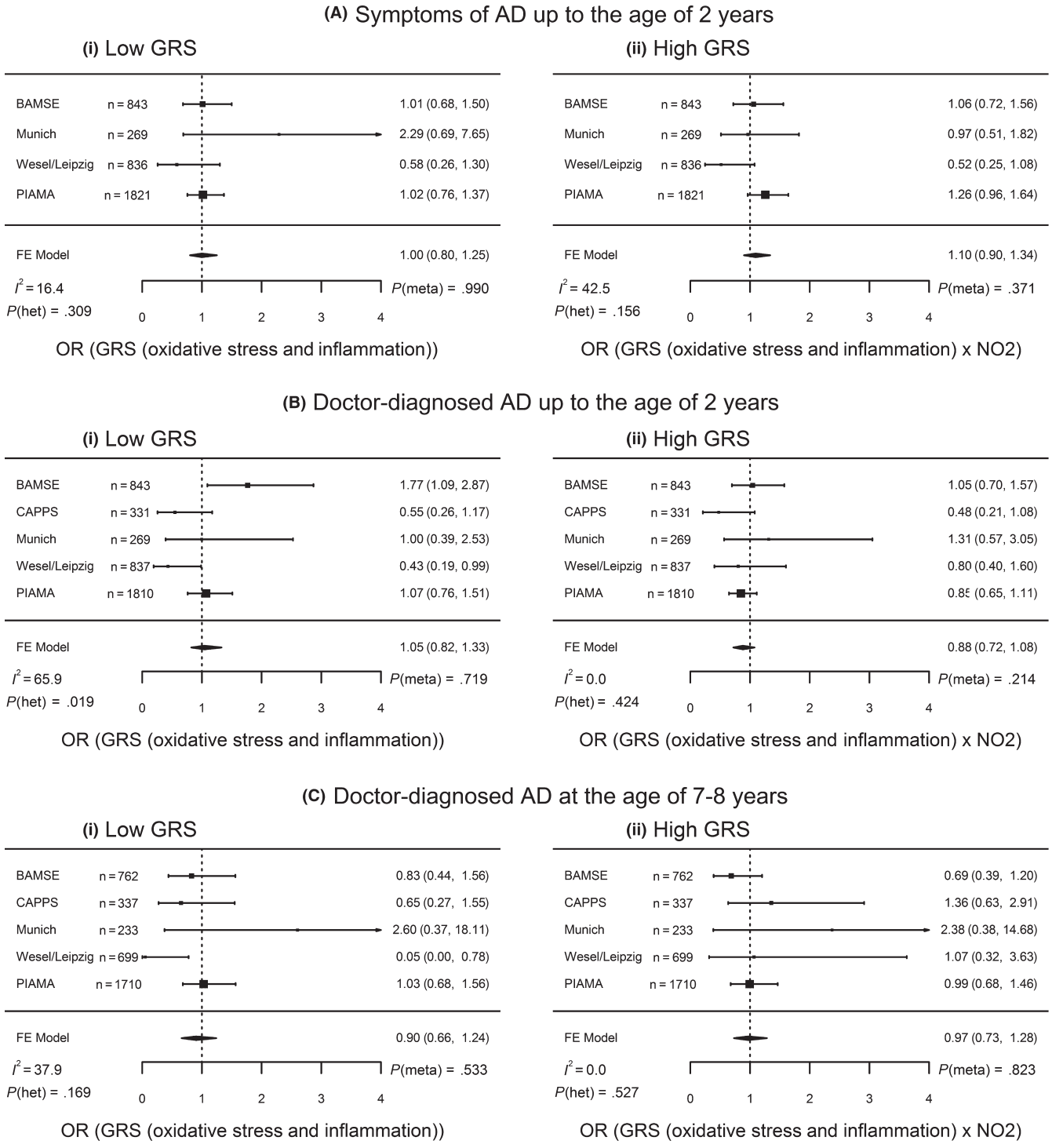

FIGURE 3 Association between NO_2 exposure at birth on atopic dermatitis (AD) in subgroups defined by a low (i) vs high (ii) weighted genetic risk score (GRS) for oxidative stress and inflammation SNPs (weights from pooled single SNPs analysis). Associations were tested within the GRS × E interaction analysis (compare Figure 2). All models were adjusted for city/center, cohort, sex, parental history of allergy, maternal smoking during pregnancy, exposure to second-hand smoke (SHS) up to the age of 2, and maternal age at childbirth. Furthermore, we considered the participation in the intervention groups as additional covariate for GINIplus, CAPPS, and PIAMA and case-control status in SAGE (asthma at the age of 7) and BAMSE (wheeze at the age of 4). OR and 95% confidence intervals are given for each cohort separately and combined by fixed-effect (FE) meta-analysis (including *P*-value (*P*(meta))). I^2 is a measure of heterogeneity between cohorts, and *P*(het) is a *P*-value for the *Q* test of heterogeneity. Munich: GINIplus/LISAplus Munich; Wesel/Leipzig: GINIplus/LISAplus Wesel/Leipzig

4.3 | Strengths and limitations

Our study has several strengths. With a sample size up to 5685, this study is the largest consortium to examine the association between TRAP and AD and its interaction with genetic variants. We focused on the traffic-related air pollutant NO_2 which is a good marker of within-city variability in exposure to traffic-related pollution.[27] Furthermore, the TAG cohorts are unique in that they have individually assigned exposures with high spatial resolution based on residential address at birth—thus capturing the important exposure window during early life. In addition, we differentiated between infantile AD (AD by the age of 2 years) and childhood AD (AD at the age of 7 or 8 years), two phenotypes that vary substantially regarding the clinical picture.[28]

A few limitations should be noted. One limitation is that the data were not collected with the use of identical strategies across all cohorts. Each cohort used different definitions of AD, which may have affected the study-specific prevalence estimates. Any misclassification of the disease outcome would likely be non-differential and would drive the results toward the null.

The panel of SNPs assessed was based on published literature describing plausible biological mechanisms and on the availability of data in at least three cohorts. Future G × E interaction studies on air pollution-induced AD might include further genetic variants that are involved in oxidative stress and inflammation, for example, null mutations in the *GSTM1* and *GSTT1* genes and two genetic determinants of infant exhaled nitric oxide (eNO) levels (rs208515 and rs1441519[29]). These SNPs were only available in GINIplus/LISAplus. In this subgroup, we found an indication for interaction with *GSTM1* null alleles (N = 1585; *P*-value(interaction) = .009; Tables S12 and S13), which further emphasizes the role of oxidative stress to air pollution-induced AD. Another approach might be to focus on genetic variants for which a marginal genetic effect was identified, namely null mutations in the *filaggrin* (*FLG*) gene or the 31 additional genotypes that have been shown to be associated with atopic AD in genomewide association studies.[30]

Although exposure estimates were individually assigned to each participant, exposure misclassification is a potential limitation because a person's true exposure is in reality a complex combination of several components.

5 | CONCLUSION

This pooled analysis of six birth cohorts does not provide evidence that TRAP increases the risk of AD in the general population. Furthermore, we found an indication that oxidative stress and inflammation are marginally associated with the prevalence of childhood AD and they may modify the susceptibility to air pollution-induced AD.

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CONFLICT OF INTEREST

Disclosure of potential conflict of interest: E. Fuertes is supported by a Marie Skłodowska-Curie Individual Fellowship (H2020-MSCA-IF-2015; proposal number 704268). C. Carlsten holds the AstraZeneca endowed Chair in Occupational and Environmental Lung Disease, and he and his work has been further supported by the AllerGen NCE and the Canada Research Chairs program, and the British Columbia Lung Association. 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 Excellence. 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. G.H. Koppelman has received grant from the Lung Foundation of the Netherlands, Ubbo Emmius Foundation, TEVA the Netherlands, outside the submitted work. The rest of the authors declare that they have no relevant conflict of interests.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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