GSTP1 and *TNF* Gene Variants and Associations between Air Pollution and Incident Childhood Asthma: The Traffic, Asthma and Genetics (TAG) Study

Elaina A. MacIntyre, Michael Brauer, Erik Melén,
Carl Peter Bauer, Mario Bauer, Dietrich Berdel, Anna Bergström,
Bert Brunekreef, Moira Chan-Yeung, Claudia Klümper,
Elaine Fuertes, Ulrike Gehring, Anna Gref, Joachim Heinrich,
Olf Herbarth, Marjan Kerkhof, Gerard H. Koppelman,
Anita L. Kozyrskyj, Göran Pershagen, Dirkje S. Postma,
Elisabeth Thiering, Carla M.T. Tiesler, and Christopher Carlsten,
for the TAG Study Group

GSTP1 and *TNF* Gene Variants and Associations between Air Pollution and Incident Childhood Asthma: The Traffic, Asthma and Genetics (TAG) Study

Elaina A. MacIntyre,[1,2] Michael Brauer,[1] Erik Melén,[3,4] Carl Peter Bauer,[5] Mario Bauer,[6] Dietrich Berdel,[7] Anna Bergström,[3] Bert Brunekreef,[8,9] Moira Chan-Yeung,[10] Claudia Klümper,[11] Elaine Fuertes,[1,2] Ulrike Gehring,[8] Anna Gref,[3] Joachim Heinrich,[2] Olf Herbarth,[12] Marjan Kerkhof,[13] Gerard H. Koppelman,[14] Anita L. Kozyrskyj,[15,16] Göran Pershagen,[3] Dirkje S. Postma,[17,18] Elisabeth Thiering,[2] Carla M.T. Tiesler,[2,19] and Christopher Carlsten,[10] for the TAG Study Group

[1]School of Population and Public Health, University of British Columbia, Vancouver, Canada; [2]Institute of Epidemiology I, Helmholtz Zentrum München, German Research Centre for Environmental Health, Neuherberg, Germany; [3]Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden; [4]Sachs' Children's Hospital, Stockholm, Sweden; [5]Department of Pediatrics, Technical University of Munich, Munich, Germany; [6]Department for Environmental Immunology, Helmholtz Centre for Environmental Research – UFZ, Leipzig, Germany; [7]Department of Pediatrics, Marien-Hospital Wesel, Wesel, Germany; [8]Institute for Risk Assessment Sciences, Utrecht University, Utrecht, the Netherlands; [9]Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht, the Netherlands; [10]Department of Medicine, University of British Columbia, Vancouver, Canada; [11] Leibniz Research Institute for Environmental Medicine, University of Düsseldorf, Düsseldorf , Germany; [12]Environmental Medicine and Hygiene, University of Leipzig, Leipzig, Germany; [13]Department of Epidemiology, University of Groningen, University Medical Center Groningen, Groningen,

the Netherlands; [14]Department of Pediatric Pulmonology and Pediatric Allergology, Beatrix Children's Hospital, University of Groningen, University Medical Center Groningen, GRIAC research Institute, Groningen, the Netherlands; [15]Department of Pediatrics, Faculty of Medicine & Dentistry, University of Alberta, Edmonton, Canada; [16]School of Public Health, University of Alberta, Edmonton, Canada; [17]University of Groningen, Department of Pulmonology, University Medical Center Groningen, Groningen, the Netherlands; [18]Groningen Research Institute for Asthma and COPD, Groningen, the Netherlands; [19]Division of Metabolic Diseases and Nutritional Medicine, Ludwig-Maximilians-University of Munich, Dr. von Hauner Children's Hospital, Munich, Germany

Address correspondence to Chris Carlsten, Vancouver General Hospital, 2775 Laurel Street, 7th Floor, Vancouver, British Columbia, V5Z1M9 Canada. Telephone: +1 (604) 875-4729. Fax: +1 (604) 875-4727. E-mail: carlsten@mail.ubc.ca

Running Title: Traffic, childhood asthma and GSTP1

Abstract

Background: Genetics may partially explain observed heterogeneity in associations between traffic-related air pollution and incident asthma.

Objective: To investigate the impact of gene variants associated with oxidative stress and inflammation on associations between air pollution and incident childhood asthma.

Methods: Traffic-related air pollution, asthma, wheeze, gene variant, and potential confounder data were pooled across six birth cohorts. Parents reported physician-diagnosed asthma and wheeze from birth to age 7-8 years (confirmed by pediatric allergist in two cohorts). Individual estimates of annual average air pollution (NO_2, $PM_{2.5}$, $PM_{2.5}$ absorbance, ozone) were assigned to each child's birth address using land-use regression, atmospheric modeling, and ambient monitoring data. Effect modification by variants in *GSTP1* (rs1138272/Ala[114]Val and rs1695/IIe[105]Val) and *TNF* (rs1800629/G-308A) was investigated.

Results: Data on asthma, wheeze, potential confounders, at least one SNP of interest and NO_2 were available for 5,115 children. *GSTP1* rs1138272 and *TNF* rs1800629 SNPs were associated with asthma and wheeze, respectively. In relation to air pollution exposure, children with ≥1 *GSTP1* rs1138272 minor allele were at increased risk of current asthma (OR=2.59; 95%CI: 1.43, 4.68 per $10\mu g/m^3$ NO_2) and ever asthma (OR=1.64; 95% CI: 1.06, 2.53) compared with homozygous major allele carriers (OR=0.95; 95% CI: 0.68, 1.32 for current and OR=1.20; 95% CI: 0.98, 1.48 for ever asthma, (Bonferroni-corrected interaction p-values 0.04 and 0.01, respectively). Similarly, for *GSTP1* rs1695, associations between NO_2 and current and ever asthma were OR=1.43 (95% CI: 1.03, 1.98) and 1.36 (95% CI: 1.08, 1.70) respectively, for minor allele carriers compared to OR=0.82 (95%CI: 0.52, 1.32) and 1.12 (95%CI: 0.84, 1.49) for

homozygous major allele carriers (Bonferroni-corrected interaction p-values 0.48 and 0.09).
There were no clear differences by *TNF* genotype.

Conclusions: Children carrying *GSTP1* rs1138272 or rs1695 minor alleles may constitute a
susceptible population at increased risk of asthma associated with air pollution.

Introduction

Evidence supporting an association between traffic-related air pollution and incident childhood asthma is growing although published estimates highlight variability across different populations. (Anderson et al. 2013; Gehring et al. 2010; Nordling et al. 2008). Genetic variation could be one explanation for this observed heterogeneity and gene-environment studies may help identify those children most susceptible to air pollution (Braback and Forsberg 2009). Large studies that combine multiple birth cohorts are ideal for examining gene-environment interactions in the development of childhood asthma, particularly where there are similar assessments of air pollution and asthma across cohorts (London 2007).

Genes related to oxidative stress are of special interest due to the biological mechanisms thought to underlie both the toxicity of traffic-related air pollution and the development of asthma (Kelly 2003). *GSTP1* codes for an enzyme that metabolizes reactive oxygen species in the lung and, despite considerable study, associations with atopic disease have not been consistent (Piacentini et al. 2013), possibly due to additional host and environment factors such as age (Salam et al. 2007). To date, *GSTP1* polymorphisms have been reported to modify the association between traffic-related air pollution and allergic sensitization (Melén et al. 2008), persistent wheeze (Schroer et al. 2009) and asthma (Carlsten et al. 2011a) in individual birth cohorts; and in one study these strata-specific findings were further attenuated by *TNF* polymorphisms (Melén et al. 2008). *TNF* mediates inflammation and is expressed in response to environmental pollutants (Wu et al. 2007). *TNF* polymorphisms have been associated with asthma (Aoki et al. 2006; Panasevich et al. 2010) and wheeze (Wu et al. 2007); and were previously reported to modify the association between ozone and asthma in healthy young adults (Yang et al. 2008).

We initiated an international collaboration, the Traffic, Asthma and Genetics Study (TAG), to examine the influence of specific candidate genes on the relationship between traffic-related air pollution and the development of childhood asthma.

Methods

The Canadian asthma primary prevention study (CAPPS) is a randomized controlled study of high-risk children (Carlsten et al. 2011b). The study of asthma, genetics and environment (SAGE) is a Canadian population-based birth cohort (Kozyrskyj et al. 2009). The children, allergy, milieu, Stockholm, epidemiological survey (BAMSE) is a population-based Swedish birth cohort (Melén et al. 2008). The German infant study on the influence of nutrition intervention plus environmental and genetic influences on allergy (GINIplus) is a population-based birth cohort (Morgenstern et al. 2008). The influence of life style factors on the development of the immune system and allergies in East and West Germany plus the influence of traffic emissions and genetics (LISAplus) study is a population-based birth cohort (Morgenstern et al. 2008). The prevention and incidence of asthma and mite allergy (PIAMA) study is a population-based birth cohort (Brunekreef et al. 2002). Detailed cohort descriptions are provided in the Supplemental Material (see Supplemental Material, Cohort Descriptions) and additional details on the TAG study have been previously published (MacIntyre et al. 2013). Parents participating in each cohort provided informed consent, and protocols were approved by all relevant ethical review boards.

Physician-diagnosed asthma and wheeze symptoms were collected from parental questionnaires. CAPPS and SAGE children were also evaluated by a pediatric allergist to confirm asthma. Current asthma and current wheeze were ascertained at 8 years (6 years for GINI/LISA wheeze;

7 years for CAPPS asthma/wheeze). Ever asthma and ever wheeze were defined using every available follow-up to 8 years. Children missing ≥ 2 follow-ups were excluded. Data were collected at slightly different time points and there were minor differences in question wording across the cohorts (MacIntyre et al. 2013).

Annual average NO_2 was estimated using traffic-related land use regression (LUR) models, except for the BAMSE cohort which used dispersion modeling (Bellander et al. 2001). LUR models developed for PIAMA and LISA/GINI Munich were based on data collected between 1999-2000 (Brauer et al. 2003), LISA/GINI Wesel in 2003, LISA/GINI Leipzig in 2004/2005 (Hochadel et al. 2006), CAPPS Vancouver in 2003 (Henderson et al. 2007), and SAGE and CAPPS Winnipeg in 2007 (Allen et al. 2010). For all study sites, integrated 14-day sampling campaigns were repeated over a 12-month period to capture seasonal patterns in air pollution. Protocols were specifically developed to spatially model long-term average pollutant concentrations. Potential predictors for traffic were screened based on their correlation with measured pollutants. Final models were assessed based on the coefficient of determination and root mean square error from cross-validation. LUR estimated $PM_{2.5}$ and $PM_{2.5}$ absorbance (a measure of elemental carbon estimated from filter reflectance measurements) were available for a subset of the combined cohort. For BAMSE, emission data for traffic-generated NOx were collected for the years 1990 and 2000; and dispersion was estimated using a dilution model based on wind speed, direction and precipitation (Nordling et al. 2008). Additionally, ozone was estimated for the European cohorts using APMoSPHERE (Beelen et al. 2009), and for CAPPS/SAGE using inverse distance weighting of the closest three ambient monitors (within 50 km). NO_2 was the only air pollution metric that was available across all six cohorts. $PM_{2.5}$, $PM_{2.5}$ absorbance and ozone were used in sensitivity analyses as estimates of exposure to these

pollutants were not available for all cohorts. Exposures were assigned for the first year of life based on geo-coded birth addresses.

Candidate genes were selected based on availability of data across all six cohorts. The following SNPs were investigated; *GSTP1* rs1138272 (merged with rs1799811; C→T: Ala[114]Val), *GSTP1* rs1695 (merged with rs947894; A→G: Ile[105]Val) and *TNF* rs1800629 (G→A: -308). Genotypic analyses were conducted at separate facilities using a variety of methods: the Canadian birth cohorts both used the Illumina BeadArray system (Illumina, San Diego, California, USA) following the manufacturer's protocol (CAPPS - peripheral blood was collected at 7 years (Carlsten et al. 2011a); SAGE - buccal samples were collected at 9 years (Kozyrskyj et al. 2009)). The PIAMA study collected buccal swabs or blood at 4/8 years and used Competitive Allele-Specific PCR using KASParTM genotyping chemistry (K-Biosciences, Herts, UK). (Koopman et al. 2002). The German studies and BAMSE used matrix-assisted laser desorption/ionization–time of flight (MALDI-TOF) mass spectrometry (Sequenom, San Diego, California, USA) following the manufacturer's protocol (GINI/LISA - blood samples were collected at 6/10 years; BAMSE – blood samples were collected at 4 years (Melén et al. 2008)). The German studies additionally used the restriction fragment length polymorphism approach for *GSTP1* rs1695 (Slama et al. 2010) and high-resolution melting-PCR using LightSNiP hybridization probe for *GSTP1* rs1138272 (TIB MolBiol, Berlin, Germany).

Data transfer and harmonization occurred in several stages. First, each cohort sent data dictionaries (translated to English, where applicable) to the main study center at the University of British Columbia. From each of the data dictionaries, a list of key variables was compiled and sent to each cohort to request data extraction. Data extracts were then prepared by each cohort

and sent to the main study center for harmonization and merging. Data were collected at different time points across the cohorts, with differences in questionnaire wording and case definitions (see MacIntyre et al. 2013 for additional information). In the pooled dataset, new variables for this analysis were derived from data common to all cohorts.

Statistical analysis

The main effect estimate for each SNP was examined using dominant genetic models. Effect modification was examined in adjusted analyses of NO_2 (per 10 μg/m^3 increase) and asthma/wheeze, stratified by genotype. Interactions between genotype and NO_2 were investigated using the log likelihood ratio for models with and without product interaction terms. Models were adjusted for potential confounders available across each cohort: study, city, gender, birth weight (< 2,500, 2,500-4,000, > 4,000 g), parental history of allergy (yes/no), maternal age at birth (≥ 35, 30-34, 25-29, ≤ 24 years), maternal smoking reported anytime during pregnancy (yes/no), environmental tobacco smoke reported in the home (yes/no), and intervention (yes/no). All pooled analyses used weighted logistic regression to adjust for inclusion of case-control data (Richardson et al. 2007). Sensitivity analyses investigated additional exposure metrics available for only a subset of pooled data ($PM_{2.5}$ (per 4 μg/m^3 increase), $PM_{2.5}$ absorbance (per 0.5 10^{-5}/m increase) and ozone (per 10 μg/m^3 increase)). All p-values were corrected for multiple testing using Bonferroni adjustment and adjusted p-values less than 0.05 were considered statistically significant. All analyses used SAS v9.2 (SAS Institute, Cary, NC).

Results

Table 1 describes the population characteristics for children included in at least one analyses (N_{total} = 5,115). The population used for TAG had a lower prevalence of low birth weight (<

2500g) babies (3% versus 12%), fewer mothers under the age of 25 years (6% versus 9%), more parents with reported atopy (55% versus 47%) and fewer mothers who smoked during pregnancy (14% versus 17%); as compared to the total recruited population for each cohort.

There were a total of 5,115 children with complete data on asthma, wheeze, potential confounders, NO_2, and at least one SNP (4,596 with *GSTP1* rs1138272, 4,748 with *GSTP1* rs1695, 4,483 with *TNF* rs1800629); 22% were part of an intervention, 52% were male, 29% were exposed to environmental tobacco smoke in the home, 55% had parental history of atopy and 19% were born to mothers 35 years of age or older. Parents of 7.5% of the children reported current asthma (ranging from 2.3-40.5% in individual cohorts), 18% reported ever asthma (4.6-41.6%), 12.5% reported current wheeze (7.0-69.0%), 45% ever wheeze (35.2-69.0%) and 7.6% reported both ever asthma and current wheeze ("active asthma"; 2.8-46.6%).

Table 2 describes pollutant distributions for NO_2, $PM_{2.5}$, $PM_{2.5}$ absorbance and ozone. Data are presented for the pooled dataset and by cohort or combinations of cohorts where an identical LUR model was used in two different cohorts (Munich, Wesel and Winnipeg). In general, the mean NO_2 concentrations for Germany and the Netherlands were similar while those for one of the Canadian locations (Winnipeg) and Sweden were lower. For the 2,743 children in sensitivity analyses, NO_2 was correlated with $PM_{2.5}$ absorbance (r = 0.72), weakly correlated with $PM_{2.5}$ (r = 0.23) and inversely correlated with ozone (r = -0.19). The inverse correlation between NO_2 and O_3 was due to the atmospheric chemistry unique to each pollutant; and the weak correlation between $PM_{2.5}$ and $PM_{2.5}$ absorbance was likely due to differences in the composition and sources of air pollution in each region.

The population distribution and number of asthma cases by genotype is presented in Table 3. All SNPs satisfied Hardy-Weinberg equilibrium based on chi-square statistics ($p < 0.05$).

Main effect estimates

Table 4 shows the main effect estimates for associations of the genotypes and air pollution exposures with the respiratory outcomes based on the pooled data for all cohorts combined. Effect estimates were calculated for homozygous and heterozygous minor allele carriers versus homozygous major allele carriers. The adjusted odds ratio for *GSTP1* rs1138272 and current asthma was 1.57 (95% CI: 1.16, 2.14; Bonferroni corrected p-value: 0.012). Findings were similar for *GSTP1* rs1138272 and ever asthma (OR = 1.49; 95% CI: 1.20, 1.84; corrected p-value: < 0.001) and current wheeze plus ever asthma (OR = 1.61; 95% CI: 1.22, 2.14; corrected p-value: 0.003). The adjusted odds ratio for *GSTP1* rs1695 and current asthma was 0.87 (95% CI: 0.68, 1.12) in pooled analysis; associations between rs1695 and remaining respiratory outcomes were consistent with the null hypothesis. The adjusted odds ratio for *TNF* rs1800629 and current asthma was 1.20 (95% CI: 0.92, 1.57). The association between ever wheeze and *TNF* rs1800629 was not statistically significant after adjusting for multiple testing (OR = 1.18; 95% CI: 1.02, 1.37; corrected p-value: 0.087). Statistically significant associations were estimated for NO_2 and ever asthma (OR = 1.23; 95% CI: 1.03-1.46 per 10 $\mu g/m^3$ increase; corrected p-value: 0.019) and for $PM_{2.5}$ and current asthma (OR = 2.08; 95% CI: 1.35-3.19 per 4 $\mu g/m^3$ increase; corrected p-value: 0.001).

Genotype stratification and interaction

The associations between NO_2 during the first year of life and respiratory outcomes, stratified by genotype, and interaction p-values are presented in Table 5 for pooled data. Effect estimates were elevated and statistically significant in carriers of minor alleles of *GSTP1* rs1138272 and

rs1695 in models for current asthma (corrected p-value for minor allele carriers: 0.006; corrected p-value for homozygous major allele carriers: 0.766; corrected p-values for interaction: 0.039) and ever asthma (corrected p-value for minor allele carriers: 0.048; corrected p-value for homozygous major allele carriers: 0.432; corrected p-values for interaction: 0.087), respectively. Overall, effect estimates were greater for minor allele carriers than for their respective homozygous major allele carriers in models for current asthma and ever asthma. Findings for *TNF* generally supported the null hypothesis.

For children who were minor allele carriers for both *GSTP1* SNPs (rs1138272 <u>and</u> rs1695; N_{total} = 728) there were statistically significant associations between NO_2 and current asthma (OR: 2.37 [95%CI: 1.31, 4.34] per $10\mu g/m^3$ NO_2; p-value = 0.005) and ever asthma (1.57 [95%CI: 1.04, 2.37]; p-value = 0.032); analyses in remaining *GSTP1* rs1138272*rs1695 strata (rs1138272 TT/TC and rs1695 AA; rs1138272 CC and rs1695 GG/GA; rs1138272 CC and rs1695 AA) were non-significant (data not shown).

Sensitivity analyses

Sensitivity analyses are presented in the Supplemental Material. The following cohorts (or cohort subsets) had assigned ozone, $PM_{2.5}$ and $PM_{2.5}$ absorbance; CAPPS Vancouver, LISA, GINI and PIAMA. There were a total of 2,743 children with complete data on asthma, wheeze, potential confounders, NO_2, ozone, $PM_{2.5}$, $PM_{2.5}$ absorbance and at least one SNP of interest (2,367 with *GSTP1* rs1138272, 2,559 with *GSTP1* rs1695, 2,318 with *TNF* rs1800629). Pooled estimates were recalculated for these additional air pollution metrics (Supplemental Material, Tables S1-S3, respectively). In analyses stratified by genotype, there was an association between ozone and current wheeze (OR = 3.67; 95% CI: 1.05, 12.7; N_{total} = 405) in *GSTP1* rs1138272 minor allele carriers; and between $PM_{2.5}$ and current asthma in all strata examined although small

13

sample size for some strata resulted in highly imprecise estimates. The results of multi-pollutant models examining NO_2 and $PM_{2.5}$, stratified by genotype, are presented in Supplemental Material, Table S4. NO_2 effect estimates were sensitive to inclusion of $PM_{2.5}$ in some analyses but these results should be interpreted cautiously as data were only available for four cohorts.

The analyses applied to the pooled data were also applied each individual cohort (Supplemental Material, Tables S5-S10). Results showed some heterogeneity in the magnitude of effect estimate but findings were generally not statistically significant due to small sample sizes. For example, the odds of current asthma per 10 $\mu g/m^3$ NO_2 for *GSTP1* rs1138272 minor allele carriers was: 2.19 (0.54, 8.86) in BAMSE, 6.33 (0.66, 60.8) in GINI and LISA Munich, 5.44 (1.23, 24.0) in GINI and LISA Wesel, 2.27 (0.69, 7.49) in PIAMA, 2.30 (0.02, 256) in CAPPS Vancouver and 19.20 (0.79, 466) in CAPPS and SAGE Winnipeg; versus 2.59 (1.43, 4.68) in pooled data. Further, the interactions between NO_2 and genotype in pooled analyses were robust in reanalyses that sequentially excluded individual cohorts (data not shown).

Finally, analyses were rerun after restricting the pooled cohort to only those children in an observational (non intervention) study arm (N_{total}=3,695; Supplemental Material, Table S11) and to children of Caucasian race (N_{total}=4,821; Supplemental Material, Table S12; data only available for CAPPS and SAGE). Results of these final sensitivities were in general agreement with findings in the full dataset.

Discussion

This study is the first Canadian-European consortium to examine the interaction between traffic-related air pollution and candidate genes of oxidative stress and inflammation in the development of childhood asthma. The pooled dataset included 381 children with current asthma and 878 with

ever asthma, and provided an unprecedented opportunity to identify populations genetically at risk for asthma development in relation to traffic-related air pollution. Our findings suggest that children with variant *GSTP1* genotypes may constitute a susceptible population at increased risk of asthma associated with air pollution.

The purpose of this work was to estimate the effect of two oxidative stress and inflammation genes on the relationship between traffic-related air pollution and the development of asthma in childhood. Asthma was associated with *GSTP1* rs1138272 in the study population. In analyses of NO_2 and respiratory outcome, stratified by genotype, minor allele carriers for *GSTP1* rs1138272 and *GSTP1* rs1695 appeared to be more susceptible to the harmful effects of air pollution versus their homozygous major allele carrying counterparts. The interaction between *GSTP1* rs1138272 and NO_2 was statistically significant in models for current asthma, ever asthma and ever wheeze.

This study utilized data from six birth cohorts. Five of these cohorts have previously reported associations between traffic-related air pollution and respiratory outcomes during childhood (Carlsten et al. 2011b; Gehring et al. 2010; Morgenstern et al. 2008; Nordling et al. 2008); and three have previously reported gene-air pollution interactions in the development of childhood asthma (Carlsten et al. 2011a; Melén et al. 2008; Nordling et al. 2008). The BAMSE cohort reported a statistically significant association between NO_x and asthma at 4 years in children who were both minor allele carriers for *GSTP1* rs1695 and homozygous major allele carriers for *TNF* rs1800629 (OR 2.9[1.0-8.2] for a $44\mu g/m^3$ increase in NO_x) (Melén et al. 2008). We did not find strong evidence for a gene-gene-environment interaction in the pooled dataset; although effect estimates were similar to those reported by Melén et al. The CAPPS study, which was particularly limited by sample size and did not have any homozygous minor allele carriers for *GSTP1* rs1138272, reported a negative but not statistically significant association between NO_2

and asthma at 7 years in children who were heterozygous for *GSTP1* rs1138272 (OR 0.76 [0.18-3.30] for a 7.2 $\mu g/m^3$ increase in NO_2; N_{Total} = 25) (Carlsten et al. 2011a). Finally, the PIAMA study has previously reported effect modification by interaction analyses for multiple *TLR2* and *TLR4* SNPs in the association between air pollution (using the same LUR models we presented) and childhood asthma in a subset of the PIAMA cohort (N_{Total} = 916; Kerkhof et al. 2010); but the current study is the first examination of *GSTP1* and *TNF*.

Polymorphisms in genes related to oxidative stress (*NQO1*, *GSTM1* and *GSTP1*) have been associated with increased risk of asthma. Previous work has reported reductions in *GSTP1* expression among rs1138272 and rs1695 minor allele carriers (Moyer et al. 2008). Minor allele carriers of rs1695 were found to be at increased risk for asthma in numerous different study populations (Tamer et al. 2004) particularly when children were exposed to environmental tobacco smoke (Palmer et al. 2006), or living in regions with high air pollution (Romieu et al. 2006), and impaired lung function was observed in schoolchildren living in areas of high urban air pollution (Reddy et al. 2012). It has also been suggested that GST genes may modify the lung response after co-exposure to diesel exhaust and allergen (Gilliland 2009). Conversely, the rs1695 SNP has been negatively associated with asthma (Aynacioglu et al. 2004; Fryer et al. 2000) and was associated with a lower risk of incident asthma with exercise in high (versus low) ozone communities (Islam et al. 2009). Few studies have had the opportunity to evaluate *GSTP1* rs1138272 and air pollution, due in part to the low frequency of minor allele carriers.

The inflammatory response gene *TNF* (rs1800629) has been reported to modify the relationship between ozone and asthma (Yang et al. 2008), and indoor home dampness and asthma (Tsai et al. 2011); however, similar to our results, no effect modification was noted in the association between traffic-related NO_2 and adult onset asthma (Castro-Giner et al. 2009).

The present study pooled data across six cohorts but it was unfortunate that of the many SNPs associated with oxidative stress and inflammation, only three were typed across all cohorts. It is possible that this study was underpowered; however, homozygous and heterozygous minor allele carriers were combined into one category for all analyses (Melén et al. 2008; Salam et al. 2007) and three of the TAG cohorts intentionally increased the proportion of children with asthma or wheeze through study design.

NO_2 is especially useful as a marker of within-city variability in exposure to traffic-related pollution and has been used as a marker of traffic pollution exposure in previous epidemiological investigations (Brunekreef 2007; Clark et al. 2010; Emenius et al. 2003; Gehring et al. 2010). Our results were not fully replicated in the subset (N = 2,743) of children with available data on additional markers of traffic-related ($PM_{2.5}$ and $PM_{2.5}$ absorbance) and regional (ozone) air pollution. The air pollution metrics examined here were considered only as markers for traffic-related air pollution and the goal of this work was not to highlight the importance of one metric over another, but instead to highlight the significant potential impact of this air pollution source.

The TAG cohorts are unique in that they have individually assigned exposures with high spatial resolution for the first year of life based on residential address at birth - thus capturing the important exposure window during early life (Clark et al. 2010; Gehring et al. 2010). Although measurements for the LUR models were not originally taken during the first year of life, detailed investigations, including two conducted in our study areas, found the spatial distribution of traffic-related pollutants to be stable for up to 12 years (Eeftens et al. 2011; Gulliver et al. 2013; Wang et al. 2012). LUR-model estimates were temporally adjusted using fixed-site monitoring data collected during the actual first year of life for cohorts that completed NO_2 monitoring campaigns more than 8 years after the birth year (CAPPS and SAGE).

An additional strength of the cohorts we included is that each recruited pregnant women or newborns and was therefore able to assess the development of asthma from birth. Ascertainment of physician-diagnosed asthma and wheeze symptoms via parental questionnaire is reasonably accurate (Burr 1992), particularly in countries with universal healthcare where lack of insurance does not hinder access to healthcare. Further, the two cohorts with the highest prevalence of asthma (SAGE and CAPPS) also completed examinations by a pediatric allergist to confirm parent reported asthma diagnoses. Further, although there were slight differences in questionnaire wording for asthma and predictors for air pollutant models, the general consistency in effect estimate direction and magnitude across analyses stratified by cohort or land use regression model (Supplemental Material, Tables S5-S10) is reassuring and further supports our findings.

The findings presented here may have been confounded by exposures associated with oxidative stress or airway inflammation. We adjusted for environmental tobacco smoke and parental history of asthma or atopic disease but it was not possible to adjust pooled analyses for the presence of allergens in the home or ethnicity. Smoking was associated with increased odds of disease in all pooled analyses; however, this finding was statistically significant only for "ever asthma" (data not shown). Sensitivity analyses excluding non-Caucasian children from the Canadian cohorts (< 80% Caucasian) were completed to ensure that our findings were not influenced by the ethnic composition of our population (Spielman et al. 2007); the proportion of Caucasian children in the European cohorts was very high (98%) and they were not restricted in the sensitivity. Finally, additional sensitivity analyses excluding children assigned to an intervention (Supplemental Material, Table S11; CAPPS, GINI and PIAMA) were completed to ensure that our findings were not impacted by preventative measures; this is particularly

important because the CAPPS study has reported a protective effect (for children assigned to the intervention) on the development of childhood asthma up to the age of seven years (Chan-Yeung et al. 2000).

Our findings add to previous research by providing further insight into the complex relationship between genetics and environmental factors in the development of childhood asthma. We found the association between traffic-related air pollution and the development of childhood asthma to be influenced somewhat by *GSTP1* polymorphisms (rs1138272 and rs1695) and these findings support the hypothesis that the oxidative stress response is important in the pathogenesis of childhood asthma, particularly in the presence of air pollution. We found no indication of effect modification by the inflammatory response gene TNF (rs1800629) on the association between traffic-related air pollution and childhood asthma. *GSTP1* (rs1138272 and rs1695) minor allele carriers may be a susceptible population at increased risk of asthma in association with air pollution.

References

Allen RW, Amram O, Wheeler A, Brauer M. 2010. The transferability of NO and NO2 land use regression models between cities and pollutants. Atmospheric Environment. 45:369-378.

Anderson HR, Favarato G, Atkinson RW. 2013. Long-term exposure to air pollution and the incidence of asthma: meta-analysis of cohort studies. Air Quality, Atmosphere and Health. 6:47-56.

Aoki T, Hirota T, Tamari M, Ichikawa K, Takeda K, Arinami T, et al. 2006. An association between asthma and TNF-308G/A polymorphism: meta-analysis. J Hum Genet. 51(8):677-685.

Aynacioglu AS, Nacak M, Filiz A, Ekinci E, Roots I. 2004. Protective role of glutathione S-transferase P1 (GSTP1) Val105Val genotype in patients with bronchial asthma. Br J Clin Pharmacol. 57(2):213-217.

Beelen R, Hoek G, Pebesma E, Vienneau D, de Hoogh K, Briggs DJ. 2009. Mapping of background air pollution at a fine spatial scale across the European Union. Sci Total Environ. 407(6):1852-1867.

Bellander T, Berglind N, Gustavsson P, Jonson T, Nyberg F, Pershagen G, et al. 2001. Using geographic information systems to assess individual historical exposure to air pollution from traffic and house heating in Stockholm. Environ Health Perspect. 109(6):633-639.

Braback L, Forsberg B. 2009. Does traffic exhaust contribute to the development of asthma and allergic sensitization in children: findings from recent cohort studies. Environ Health. 8:17.

Brauer M, Hoek G, van Vliet P, Meliefste K, Fischer P, Gehring U, et al. 2003. Estimating long-term average particulate air pollution concentrations: application of traffic indicators and geographic information systems. Epidemiology. 14(2):228-239.

Brunekreef B, Smit J, de Jongste J, Neijens H, Gerritsen J, Postma D, et al. 2002. The prevention and incidence of asthma and mite allergy (PIAMA) birth cohort study: design and first results. Pediatr Allergy Immunol. 13 Suppl 15:55-60.

Brunekreef B. 2007. Health effects of air pollution observed in cohort studies in Europe. J Expo Sci Environ Epidemiol. 17 Suppl 2:S61-S65.

Burr ML. 1992. Diagnosing asthma by questionnaire in epidemiological surveys. Clin Exp Allergy. 22(5):509-510.

Carlsten C, Dybuncio A, Becker A, Chan-Yeung M, Brauer M. 2011a. GSTP1 polymorphism modifies risk for incident asthma associated with nitrogen dioxide in a high-risk birth cohort. Occup Environ Med. 68(4):308.

Carlsten C, Dybuncio A, Becker A, Chan-Yeung M, Brauer M. 2011b. Traffic-related air pollution and incident asthma in a high-risk birth cohort. Occup Environ Med. 68(4):291-295.

Castro-Giner F, Kunzli N, Jacquemin B, Forsberg B, de Cid R, Sunyer J, et al. 2009. Traffic-related air pollution, oxidative stress genes, and asthma (ECHRS). Environ Health Perspect. 117(12):1919-1924.

Chan-Yeung M, Manfreda J, Dimich-Ward H, Ferguson A, Watson W, Becker A. 2000. A randomized controlled study on the effectiveness of a multifaceted intervention program in the primary prevention of asthma in high-risk infants. Arch. Pediatr. Adolesc. Med. 154(7):657–663.

Clark NA, Demers PA, Karr CJ, Koehoorn M, Lencar C, Tamburic L, et al. 2010. Effect of early life exposure to air pollution on development of childhood asthma. Environ Health Perspect. 118(2):284-290.

Eeftens M, Beelen R, Fischer P, Brunekreef B, Meliefste K, Hoek G. 2011. Stability of measured and modelled spatial contrasts in NO(2) over time. Occup Environ Med. 68(10):765-770.

Emenius G, Pershagen G, Berglind N, Kwon HJ, Lewne M, Nordvall SL, et al. 2003. NO2, as a marker of air pollution, and recurrent wheezing in children: a nested case-control study within the BAMSE birth cohort. Occup Environ Med. 60(11):876-881.

Fryer AA, Bianco A, Hepple M, Jones PW, Strange RC, Spiteri MA. 2000. Polymorphism at the glutathione S-transferase GSTP1 locus. A new marker for bronchial hyperresponsiveness and asthma. Am J Respir Crit Care Med. 161(5):1437-1442.

Gehring U, Wijga AH, Brauer M, Fischer P, de Jongste JC, Kerkhof M, et al. 2010. Traffic-related air pollution and the development of asthma and allergies during the first 8 years of life. Am J Respir Crit Care Med. 181(6):596-603.

Gilliland FD. 2009. Outdoor air pollution, genetic susceptibility, and asthma management: opportunities for intervention to reduce the burden of asthma. Pediatrics. Suppl 3:S168-S173.

Gulliver J, de Hoogh K, Hansell A, Vienneau D. Development and back-extrapolation of NO2 land use regression models for historic exposure assessment in Great Britain. Environ Sci Technol. 2013 Jul 16;47(14):7804-11.

Henderson SB, Beckerman B, Jerrett M, Brauer M. 2007. Application of land use regression to estimate ambient concentrations of traffic-related NOX and fine particulate matter. Environ Sci Tech. 41(7):2422-2428.

Hochadel M, Heinrich J, Gehring U, Morgenstern V, Kuhlbusch T, Link E, et al. 2006. Predicting long-term average concentrations of traffic-related air pollutants using GIS-based information. Atmos Environ. 40:542-553.

Islam T, Berhane K, McConnell R, Gauderman WJ, Avol E, Peters JM, et al. 2009. Glutathione-S-transferase (GST) P1, GSTM1, exercise, ozone and asthma incidence in school children. Thorax. 64(3):197-202.

Kelly FJ. 2003. Oxidative stress: its role in air pollution and adverse health effects. Occup Environ Med. 60(8):612-616.

Kerkhof M, Postma DS, Brunekreef B, Reijmerink NE, Wijga AH, de Jongste JC, et al. 2010. Toll-like receptor 2 and 4 genes influence susceptibility to adverse effects of traffic-related air pollution on childhood asthma. Thorax. 65(8):690-697.

Koopman LP, van Strien RT, Kerkhof M, Wijga A, Smit HA, de Jongste JC, et al. 2002 Placebo-controlled trial of house dust mite-impermeable mattress covers: effect on symptoms in early childhood. Am J Respir Crit Care Med. Aug 1;166(3):307-313.

Kozyrskyj AL, HayGlass KT, Sandford AJ, Pare PD, Chan-Yeung M, Becker AB. 2009. A novel study design to investigate the early-life origins of asthma in children (SAGE study). Allergy. 64(8):1185-1193.

London SJ. 2007. Gene-air pollution interactions in asthma. Proc Am Thorac Soc. 4(3):217-220.

MacIntyre EA, Carlsten C, MacNutt M, Fuertes E, Melén E, Tiesler CMT, et al. 2013. Traffic, asthma and genetics: combining international birth cohort data to examine genetics as a mediator of traffic-related air pollution's impact on childhood asthma. Eur J Epi. 2013;27(7):597-606..

Melén E, Nyberg F, Lindgren CM, Berglind N, Zucchelli M, Nordling E, et al. 2008. Interactions between glutathione S-transferase P1, tumor necrosis factor, and traffic-related air pollution for development of childhood allergic disease. Environ Health Perspect. 116(8):1077-1084.

Morgenstern V, Zutavern A, Cyrys J, Brockow I, Koletzko S, Kramer U, et al. 2008. Atopic diseases, allergic sensitization, and exposure to traffic-related air pollution in children. Am J Respir Crit Care Med. 177(12):1331-1337.

Moyer AM, Salavaggione OE, Wu TY, Moon I, Eckloff BW, Hildebrandt MA, et al. 2008. Glutathione s-transferase p1: gene sequence variation and functional genomic studies. Cancer Res. 68(12):4791-4801.

Nickel R, Haider A, Sengler C, Lau S, Niggemann B, Deichmann KA, et al. 2005. Association study of Glutathione S-transferase P1 (GSTP1) with asthma and bronchial hyper-responsiveness in two German pediatric populations. Pediatr Allergy Immunol. 16(6):539-541.

Nordling E, Berglind N, Melen E, Emenius G, Hallberg J, Nyberg F, et al. 2008. Traffic-related air pollution and childhood respiratory symptoms, function and allergies. Epidemiology. 19(3):401-408.

Palmer CN, Doney AS, Lee SP, Murrie I, Ismail T, Macgregor DF, et al. 2006. Glutathione S-transferase M1 and P1 genotype, passive smoking, and peak expiratory flow in asthma. Pediatrics. 118(2):710-716.

Panasevich S, Lindgren C, Kere J, Wickman M, Pershagen G, Nyberg F, et al. 2010. Interaction between early maternal smoking and variants in TNF and GSTP1 in childhood wheezing. Clin Exp Allergy. 40(3):458-467.

Piacentini S, Polimanti R, Simonelli I, Donno S, Pasqualetti P, Manfellotto D, et al. 2013. Glutathione S-transferase polymorphisms, asthma susceptibility and confounding variables: a meta-analysis. Mol Biol Rep. 40(4):3299-313.

Reddy P, Naidoo RN, Robins TG, Mentz G, Li H, London SJ, et al. 2012. GSTM1 and GSTP1 gene variants and the effect of air pollutants on lung function measures in South African children. Am J Ind Med. 55(12):1078-1086.

Richardson DB, Rzehak P, Klenk J, Weiland SK. 2007. Analyses of case-control data for additional outcomes. Epidemiology. 18(4):441-445.

Romieu I, Ramirez-Aguilar M, Sienra-Monge JJ, Moreno-Macias H, del Rio-Navarro BE, David G, et al. 2006. GSTM1 and GSTP1 and respiratory health in asthmatic children exposed to ozone. Eur Respir J. 28(5):953-959.

Salam MT, Lin PC, Avol EL, Gauderman WJ, Gilliland FD. 2007. Microsomal epoxide
hydrolase, glutathione S-transferase P1, traffic and childhood asthma. Thorax. 62(12):1050-
1057.

Schroer KT, Biagini Myers JM, Ryan PH, LeMasters GK, Bernstein DI, Villareal M, et al. 2009.
Associations between multiple environmental exposures and Glutathione S-Transferase P1
on persistent wheezing in a birth cohort. J Pediatr. 154(3):401-408, 8 e1.

Slama R, Grabsch C, Lepeule J, Siroux V, Cyrys J, Sausenthaler S, et al. 2010 Maternal fine
particulate matter exposure, polymorphism in xenobiotic-metabolizing genes and offspring
birth weight. Reprod Toxicol. Dec;30(4):600-612.

Spielman RS, Bastone LA, Burdick JT, Morley M, Ewens WJ, Cheung VG. 2007. Common
genetic variants account for differences in gene expression among ethnic groups. Nat Genet.
39(2):226-231.

Tamer L, Calikoglu M, Ates NA, Yildirim H, Ercan B, Saritas E, et al. 2004. Glutathione-S-
transferase gene polymorphisms (GSTT1, GSTM1, GSTP1) as increased risk factors for
asthma. Respirology. 9(4):493-498.

Tsai CH, Tung KY, Chen CH, Lee YL. 2011. Tumour necrosis factor G-308A polymorphism
modifies the effect of home dampness on childhood asthma. Occup Environ Med.
68(10):771-776.

Wang R, Henderson SB, Sbihi H, Allern RW, Brauer M. 2012. Temporal Stability of Land Use
Regression Models for Traffic-Related Air Pollution. Atmos Environ. 64:312-319.

Wu H, Romieu I, Sienra-Monge JJ, del Rio-Navarro BE, Anderson DM, Dunn EW, et al. 2007.
Parental smoking modifies the relation between genetic variation in tumor necrosis factor-
alpha (TNF) and childhood asthma. Environ Health Perspect. 115(4):616-622.

Yang IA, Fong KM, Zimmerman PV, Holgate ST, Holloway JW. 2008. Genetic susceptibility to
the respiratory effects of air pollution. Thorax. 63(6):555-563.

Table 1. Population characteristics for the pooled TAG dataset and for each individual cohort.

Characteristic	Pooled	BAMSE	CAPPS	GINI	LISA	SAGE	PIAMA
Total population	5115 (100)	912 (100)	346 (100)	1126 (100)	697 (100)	178 (100)	1856 (100)
Intervention	1114 (21.8)	n/a	181 (52.3)	528 (46.9)	n/a	n/a	405 (21.8)
Male gender	2661 (52.0)	484 (53.1)	189 (54.6)	584 (51.9)	361 (51.8)	96 (53.9)	947 (51.0)
Birth weight							
<2500g	150 (2.9)	45 (4.9)	16 (4.6)	19 (1.7)	0 (-)	13 (7.3)	57 (3.1)
2500-4000g	4085 (79.9)	706 (77.4)	256 (74.0)	952 (84.6)	587 (84.2)	132 (74.2)	1452 (78.2)
>4000g	880 (17.2)	161 (17.6)	74 (21.4)	155 (13.8)	110 (15.8)	33 (18.5)	347 (18.7)
Maternal smoking during pregnancy	688 (14.1)	119 (13.1)	29 (8.4)	149 (15.7)	91 (13.6)	18 (10.2)	282 (15.3)
Environmental tobacco smoke at home[a]	1465 (28.6)	164 (18.0)	71 (20.5)	315 (28.0)	158 (22.7)	34 (19.1)	723 (39.0)
Parental atopy	2802 (54.8)	530 (58.1)	319 (92.2)	459 (40.8)	348 (49.9)	126 (70.8)	1020 (55.0)
Mother's age at birth							
≥ 35 years	978 (19.1)	189 (20.7)	112 (32.4)	215 (19.1)	132 (18.9)	28 (15.7)	302 (16.3)
30 – 34 years	2278 (44.5)	363 (40.2)	125 (36.1)	553 (49.1)	306 (44.1)	74 (41.6)	857 (46.2)
25 – 29 years	1574 (30.8)	287 (31.5)	86 (24.9)	314 (27.9)	221 (31.7)	54 (30.3)	612 (33.0)
≤ 24 years	285 (5.6)	73 (8.0)	23 (6.7)	44 (3.9)	38 (5.5)	22 (12.4)	85 (4.5)
Current asthma	381 (7.5)	112 (12.3)	62 (17.9)	42 (3.7)	16 (2.3)	72 (40.5)	77 (4.2)
Ever asthma	878 (17.7)	272 (29.8)	144 (41.6)	86 (8.2)	30 (4.6)	72 (40.5)	274 (15.0)
Current wheeze	623 (12.5)	162 (17.9)	76 (22.0)	112 (10.1)	63 (9.4)	80 (69.0)	130 (7.0)
Ever wheeze	2243 (44.9)	535 (58.7)	159 (46.0)	383 (35.2)	278 (40.0)	80 (69.0)	808 (44.0)
Ever asthma AND current wheeze	372 (7.6)	122 (13.5)	61 (17.3)	41 (3.9)	18 (2.8)	54 (46.6)	76 (4.2)

[a] Defined as one or more person in the household regularly smoking indoors during the first year of life (exceptions: 2nd year of life in GINI; 8th year of life in SAGE). n/a – not applicable.

Table 2. Annual average pollutant statistics for the first year of life for pooled data and for each individual model.

Statistic	Pooled Data	Sweden (BAMSE)	Munich, Germany (LISA/GINI)	Wesel and Leipzig, Germany (LISA/GINI)	The Netherlands (PIAMA)	Vancouver, Canada (CAPPS)	Winnipeg, Canada (CAPPS/SAGE)
NO_2 ($\mu g/m^3$)							
Mean (SD)	22.7 (8.1)	16.7 (9.1)	27.5 (6.3)	22.9 (3.5)	24.6 (7.4)	32.5 (6.0)	12.2 (3.6)
IQR	10.3	13.8	8.1	4.9	11.2	7.1	5.2
Range	2.2-66.8	2.2-49.9	19.5-66.9	13.9-41.4	12.6-58.4	18.9-55.1	4.1-21.5
Ozone ($\mu g/m^3$)							
Mean (SD)	36.8 (6.5)	n/a	43.9 (2.1)	39.4 (3.6)	33.8 (4.7)	21.2 (2.8)	n/a
IQR	9.1	n/a	1.0	6.9	4.3	4.8	n/a
Range	13.3-55.9	n/a	38.8-55.9	32.3-48.6	13.3-47.3	13.4-27.0	n/a
$PM_{2.5}$ ($\mu g/m^3$)							
Mean (SD)	15.2 (3.4)	n/a	13.3 (1.3)	n/a	16.7 (1.9)	5.6 (2.6)	n/a
IQR	4.1	n/a	1.5	n/a	3.4	3.9	n/a
Range	0.0-25.1	n/a	11.9-21.9	n/a	13.5-25.2	0.0-10.0	n/a
$PM_{2.5}$ Absorbance ($10^{-5}/m$)							
Mean (SD)	1.7 (0.4)	n/a	1.7 (0.3)	1.6 (0.2)	1.7 (0.4)	1.6 (1.2)	n/a
IQR	0.4	n/a	0.3	0.2	0.6	1.1	n/a
Range	0.0-5.0	n/a	1.4-4.3	0.8-2.3	0.8-3.7	0.0-5.0	n/a

SD – standard deviation; IQR – interquartile range; n/a – not applicable.

Table 3. Population distribution by minor allele carriers and homozygous major allele carriers for *GSTP1* and *TNF* (pooled data).

SNP Genotype	Number of children	Number of current asthma cases	Number of children in the highest tertile of NO_2	Children with current asthma in the highest tertile of NO_2
GSTP1 **rs1138272**				
TT/TC	789	80 (10.1%)	224	15 (6.7%)
CC	3,807	287 (7.5%)	1,144	57 (5.0%)
GSTP1 **rs1695**				
GG/GA	2,740	199 (7.3%)	873	34 (3.9%)
AA	2,008	170 (8.5%)	618	39 (6.3%)
TNF **rs1800629**				
AA/AG	1,411	119 (8.4%)	421	31 (7.4%)
GG	3,072	244 (7.9%)	917	40 (4.4%)

Table 4. Main genetic and environmental effect estimates for *GSTP1, TNF,* and NO_2 for asthma and wheeze at school age (pooled data).

Exposure	Current asthma			Ever asthma			Current wheeze			Ever wheeze			Ever asthma and current wheeze		
	N	aOR[a] (95%CI)	p-val[b]	N	aOR[a] (95%CI)	p-val[b]	N	aOR[a] (95%CI)	p-val[b]	N	aOR[a] (95%CI)	p-val[b]	N	aOR[a] (95%CI)	p-val[b]
GSTP1 rs1138272 TT/TC v. CC	4596	1.57 (1.16, 2.14)	0.012	4465	1.49(1.20, 1.84)	<0.001	4483	1.15(0.84, 1.57)	0.560	4487	1.01(0.84, 1.22)	0.918	4377	1.61(1.22, 2.14)	0.003
GSTP1 rs1695 GG/GA v. AA	4748	0.87 (0.68, 1.12)	0.285	4635	0.91(0.77, 1.08)	0.430	4659	1.02(0.81, 1.29)	0.860	4633	0.96(0.83, 1.10)	0.803	4547	0.94(0.75, 1.19)	0.628
TNF rs1800629 AA/AG v. GG	4483	1.20(0.92, 1.57)	0.258	4356	1.04(0.87, 1.26)	0.647	4371	1.22(0.95, 1.56)	0.345	4373	1.18(1.02, 1.37)	0.087	4267	1.10(0.85, 1.41)	0.628
Traffic-related NO_2 (per $10\mu g/m^3$)	5115	1.14(0.98, 1.48)	0.322	4965	1.23(1.03, 1.46)	0.019	4999	1.12(0.89, 1.40)	0.337	4995	1.04(0.91, 1.18)	0.610	4874	1.13(0.87, 1.46)	0.374
Ozone (per $10\mu g/m^3$)	2743	0.81(0.51, 1.27)	0.351	2671	0.86(0.66, 1.13)	0.277	2728	1.29(0.88, 1.88)	0.197	2704	0.90(0.74, 1.10)	0.318	2659	1.01(0.62, 1.67)	0.960
Traffic-related $PM_{2.5}$ (per $4\mu g/m^3$)	2743	2.08(1.35, 3.19)	0.001	2671	1.17(0.86, 1.60)	0.323	2728	1.33(0.90, 1.95)	0.147	2704	0.95(0.74, 1.22)	0.687	2659	1.46(0.93, 2.32)	0.102
Traffic-related $PM_{2.5}$ absorbance (per 0.5 10^{-5}/m)	2743	1.07(0.91, 1.26)	0.441	2671	1.06(0.95, 1.19)	0.283	2728	1.06(0.93, d1.21)	0.395	2704	1.00 (0.91, 1.10)	0.963	2659	1.07(0.92, 1.25)	0.369

[a]Adjusted for study, city, intervention, gender, maternal age at birth, maternal smoking during pregnancy, environmental tobacco smoke in the home, birth weight and parental atopy. [b]P-values were corrected for multiple testing using the Bonferroni method.

Table 5. Association between traffic-related NO_2 during the first year of life and asthma and wheeze at school age (pooled data).

Gene	Current asthma			Ever asthma			Current wheeze			Ever wheeze			Ever asthma and current wheeze		
	N	aOR[a] (95%CI)	p-val[b]	N	aOR[a] (95%CI)	p-val[b]	N	aOR[a] (95%CI)	p-val[b]	N	aOR[a] (95%CI)	p-val[b]	N	aOR[a] (95%CI)	p-val[b]
GSTP1 **rs1138272**															
TT/TC	789	2.59(1.43, 4.68)	0.006	769	1.64(1.06, 2.53)	0.075	772	1.26(0.71, 2.23)	0.821	775	1.17(0.82, 1.65)	0.467	757	1.30(0.67, 2.52)	0.669
CC	3807	0.95(0.68, 1.32)	0.766	3696	1.20(0.98, 1.48)	0.140	3711	1.09(0.82, 1.45)	0.821	3712	1.09(0.92, 1.29)	0.467	3620	1.17(0.85, 1.61)	0.669
Interaction[c]			0.039			0.012			0.096			0.024			0.180
GSTP1 **rs1695**															
GG/GA	2704	[c]1.43(1.03, 1.98)	0.096	2677	1.36(1.08, 1.70)	0.048	2695	1.18(0.86, 1.60)	0.821	2692	1.11(0.93, 1.33)	0.467	2632	1.16(0.82, 1.65)	0.669
AA	2008	0.82(0.52, 1.32)	0.626	1958	1.12(0.84, 1.49)	0.432	1964	1.03(0.72, 1.46)	0.888	1951	0.98(0.79, 1.23)	0.886	1915	1.09(0.70, 1.68)	0.709
Interaction[c]			0.484			0.087			0.187			0.026			0.436
TNF **rs1800629**															
AA/AG	1411	1.30(0.85, 2.00)	0.448	1372	1.29(0.94, 1.78)	0.143	1383	1.04(0.68, 1.59)	0.888	1384	1.14(0.86, 1.49)	0.467	1350	1.13(0.70, 1.82)	0.709
GG	3072	1.07(0.72, 1.57)	0.766	2984	1.21(0.97, 1.52)	0.140	2988	1.24(0.92, 1.68)	0.821	2989	1.09(0.91, 1.30)	0.467	2917	1.15(0.81, 1.63)	0.669
Interaction[c]			0.210			0.345			0.096			0.337			0.436

[a]For a 10 µg/m^3 increase in NO_2. Adjusted for study, city, intervention, gender, maternal age at birth, maternal smoking during pregnancy, environmental tobacco smoke in the home, birth weight and parental atopy. [b] P-values were corrected for multiple testing using the Bonferroni method. [c]P-values for interaction were calculated based on the likelihood-ratio tests between models with and without interaction terms and were corrected for multiple testing using the Bonferroni method