AJRCCM Articles in Press. Published on 30-November-2016 as 10.1164/rccm.201605-1026OC

Genome-wide interaction analysis of air pollution exposure and childhood asthma with functional follow-up

Anna Gref,[1] Simon Kebede Merid,[1] Olena Gruzieva,[1] Stéphane Ballereau,[2] Allan Becker,[3] Tom Bellander,[1] Anna Bergström,[1] Yohan Bossé,[4] Matteo Bottai,[1] Moira Chan-Yeung,[5] Elaine Fuertes,[6,7] Despo Ierodiakonou,[8,9] Ruiwei Jiang,[10] Stéphane Joly,[2] Meaghan Jones,[10] Michael S. Kobor,[10] Michal Korek,[1] Anita L. Kozyrskyj,[11] Ashish Kumar,[1,29] Nathanaël Lemonnier,[2] Elaina MacIntyre,[6,7,12] Camille Ménard,[2] David Nickle,[13] Ma'en Obeidat,[14] Johann Pellet,[2] Marie Standl,[6] Annika Sääf,[1] Cilla Söderhäll,[15,30] Carla MT. Tiesler,[6,16] Maarten van den Berge,[17,18] Judith M. Vonk,[9,18] Hita Vora,[19] Cheng-Jian Xu,[17,18,20] Josep M. Antó, [21] Charles Auffray,[2] Michael Brauer,[22] Jean Bousquet,[23] Bert Brunekreef,[24] W. James Gauderman,[19] Joachim Heinrich,[6] Juha Kere,[15] Gerard H. Koppelman,[18,25] Dirkje Postma,[18,26] Christopher Carlsten,[27] Göran Pershagen,[1] Erik Melén,[1,28].

[1]Institute of Environmental Medicine, Karolinska Institutet and Centre for Occupational and Environmental Medicine, Stockholm County Council, Stockholm, Sweden; [2]European Institute for Systems Biology and Medicine, CNRS-ENS-UCBL, Université de Lyon, Lyon, France; [3]Department of Pediatrics and Child Health, University of Manitoba, Winnipeg, Manitoba, Canada; [4]Institut universitaire de cardiologie et de pneumologie de Québec, Department of Molecular Medicine, Laval University, Quebec, Canada; [5]Department of Medicine, University of British Columbia, Vancouver, British Columbia, Canada; [6]Institute of Epidemiology I, Helmholtz Zentrum München – German Research Centre for Environmental Health, Neuherberg, Germany; [7]School of Population and Public Health, University of British Columbia, Vancouver, British Columbia, Canada; [8]Section of Paediatrics, Department of Medicine, Imperial College

London, London, United Kingdom; [9]University of Groningen, University Medical Center Groningen, Department of Epidemiology, Groningen, the Netherlands; [10]Centre for Molecular Medicine and Therapeutics, Child and Family Research Institute, Department of Medical Genetics, University of British Columbia, Vancouver, British Columbia, Canada; [11]Department of Pediatrics, University of Alberta, Edmonton, Alberta, Canada; [12]Environmental and Occupational Health, Public Health Ontario, Toronto, Ontario, Canada; [13]Merck & Co Inc, Rahway, New Jersey, USA; [14]Centre for Heart Lung Innovation, University of British Columbia, Vancouver, British Columbia, Canada; [15]Department of Biosciences and Nutrition, and Center for Innovative Medicine (CIMED), Karolinska Institutet, Stockholm, Sweden; [16]Division of Metabolic Diseases and Nutritional Medicine, Ludwig-Maximilians-University of Munich, Dr. von Hauner Children's Hospital, Munich, Germany; [17]University of Groningen, University Medical Center Groningen, Department of Pulmonology, GRIAC research institute, Groningen, the Netherlands; [18]University of Groningen, University Medical Center Groningen, Groningen Research Institute for Asthma and COPD, GRIAC Research Institute, Groningen, the Netherlands; [19]Preventive Medicine, University of Southern California, Los Angeles, California, USA; [20]University of Groningen, University Medical Center Groningen, Department of Genetics, GRIAC research institute, Groningen, the Netherlands; [21]Centre for Research in Environmental Epidemiology, Barcelona, Spain; [22]School of Population and Public Health, University of British Columbia, Vancouver, British Columbia, Canada; [23]CHU Montpellier, University of Montpellier, Montpellier, France; [24]Institute for Risk Assessment Sciences, Universiteit Utrecht, Utrecht, the Netherlands; [25]University of Groningen, University Medical Center Groningen, Pediatric Pulmonology and Pediatric Allerogology, Beatrix Children’s Hospital, GRIAC Research Institute, Groningen, the Netherlands; [26]University of Groningen,

AJRCCM Articles in Press. Published on 30-November-2016 as 10.1164/rccm.201605-1026OC

University Medical Center Groningen, Department of Pulmonary Medicine and Tuberculosis, Groningen, the Netherlands; [27]Department of Medicine, The University of British Columbia, Vancouver, British Columbia, Canada; [28]Sachs Children's Hospital, Stockholm, Sweden. [29]Department of Public Health Epidemiology, Unit of Chronic Disease Epidemiology, Swiss Tropical and Public Health Institute, University of Basel, Switzerland; [30]Department of Women´s and Children´s Health, Karolinska Institutet, Stockholm, Sweden

Corresponding author

Erik Melén, PhD

Institute of Environmental Medicine, Karolinska Institutet, BOX 210, 17177 Stockholm, Sweden

Phone: +46 852487508

Fax: +46 8524304571

Email: erik.melen@ki.se

Author contributions

EM, GP, CC and MB designed the study. M Bottai provided statistical competence in the study design. A Bergström, GP and EM coordinated BAMSE. TB and GP coordinated exposure assessment in BAMSE, and OG and MK performed exposure assessment. AK performed imputation of the BAMSE gwas data. AG performed gwis statistical analysis in BAMSE and CAPPS/SAGE and meta-analysis. AS provided knowledge in statistical analysis of BAMSE gwis data. JH coordinated GINI and LISA. EF performed exposure assessment in GINI/LISA. CMTT performed imputation of the GINI/LISA gwas data and MS performed statistical analysis.

BB, GHK and DP coordinated PIAMA. JMV contributed with PIAMA data. DI performed statistical analysis in PIAMA. WJG coordinated CHS. HV performed statistical analysis in CHS. MCY coordinated CAPPS. A Becker and CC coordinated CAPPS and SAGE. MB coordinated exposure assessment in CAPPS/SAGE and E MacIntyre and MB performed exposure data linkage. ALK contributed with CAPPS and SAGE data. JK coordinated methylation analysis in BAMSE. CS provided BAMSE methylation data, SKM, AK and OG performed QC, and AG and OG performed methylation analysis. CJX and GHK provided methylation QC and analysis protocol. CC coordinated the short term diesel exhaust exposure study, MSK coordinated the methylation analysis, RJ and MJ performed statistical analysis. DP coordinated lung eQTL data. MVDB provided lung eQTL data from Groningen and performed statistical analysis of lung eQTL data. YB provided lung eQTL data from Quebec City and MO provided lung eQTL data from Vancouver. DN oversaw genotyping and gene expression measurements for the lung eQTL dataset. JMA and JB coordinated the MeDALL data. SB, NL, JP, SJ, CM and CA provided transcriptomics data for BAMSE. SKM performed QC and statistical analysis.
All authors were involved in data interpretation and drafting of the manuscript.

Funding sources: BAMSE was supported by The Swedish Research Council to GP and EM, The Swedish Heart-Lung Foundation, Freemason Child House Foundation in Stockholm, Centre for Allergy Research to EM, Stockholm County Council (ALF), the Strategic Research Programme (SFO) in Epidemiology at Karolinska Institutet to EM, MeDALL (Mechanisms of the Development of ALLergy) a collaborative project conducted within the European Union (grant agreement No. 261357; JB and JMA coordinators), Swedish foundation for strategic research (SSF RBc08-0027) to JK, The Swedish Research Council Formas to GP and the

AJRCCM Articles in Press. Published on 30-November-2016 as 10.1164/rccm.201605-1026OC

Swedish Environment Protection Agency to GP and TB. GINI and LISA had personal and financial support by the Munich Center of Health Sciences (MCHEALTH) as part of the Ludwig-Maximilians University Munich LMU innovative to JH. The PIAMA study was funded by grants from the Dutch Asthma Foundation (grant 3.4.01.26, 3.2.06.022, 3.4.09.081 and 3.2.10.085CO), the ZON-MW Netherlands Organization for Health Research and Development (grant 912-03-031), the Stichting Astmabestrijding and the Ministry of the Environment. Genome-wide genotyping was funded by the European Commission as part of GABRIEL (A multidisciplinary study to identify the genetic and environmental causes of asthma in the European Community) contract number 018996 under the Integrated Program LSH-2004-1.2.5-1 Post genomic approaches to understand the molecular basis of asthma aiming at a preventive or therapeutic control to DP, BB and GHK. The Children's Health Study has been supported in part by grants from the National Institute of Environmental Health Sciences (Grant numbers ES #011627, #07048 and #022719) and the National Heart Lung and Blood Institute (Grant numbers HL #087680) to WJG. The CAPPS was supported by the Canadian Institutes of Health Research, the British Columbia Lung Association, and the Manitoba Medical Service Foundation. The SAGE was supported by the Canadian Institutes of Health Research. The ''Traffic Asthma and Genetics (TAG)'' collaboration was supported by the AllerGen Networks of Centres of Excellence to MB, CC, AB, AK. The lung eQTL study at Laval University was supported by the Chaire de pneumologie de la Fondation JD Bégin de l'Université Laval, the Fondation de l'Institut universitaire de cardiologie et de pneumologie de Québec, the Respiratory Health Network of the FRQS, the Canadian Institutes of Health Research (MOP-123369), and the Cancer Research Society and Read for the Cure to YB. The CNRS and the FP7-MeDALL Consortium (Mechanisms of the Development of Allergy, Grant Agreement FP7 No.264357) to

NL, JP, SB and CA. YB is the recipient of a Junior 2 Research Scholar award from the Fonds de recherche Québec – Santé (FRQS). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Role of the funding source: Funding sources had no role in the study design, in the collection, analysis, and interpretation of data, in the writing of the report, and in the decision to submit the paper for publication.

Conflict of declaration statement: The authors declare no conflict of interest.

Running title: GWIS, traffic air pollution and asthma

Character count running title: 38/50

Descriptor number: 6.6 Gene-Environment Interaction

Word count main text: 4050/3500

At a Glance Commentary

Scientific Knowledge on the Subject: Air pollution exposure early in life has been associated with asthma, but the mechanisms behind this effect are largely unknown. Understanding the biological mechanism that connects air pollutants with asthma and respiratory diseases has the potential to point to new targets for therapeutic intervention and to identify susceptible subgroups in the population.

What This Study Adds to the Field: We performed a genome-wide interaction study followed by functional genomics analyses that indicated involvement of several genes at the genomic,

AJRCCM Articles in Press. Published on 30-November-2016 as 10.1164/rccm.201605-1026OC

epigenomic and transcriptomic levels for asthma related to air pollution exposure. Our results support the notion that gene-environment interactions are important for asthma development.

This article has an online data supplement, which is accessible from this issue's table of content online at www.atsjournals.org

Abstract

Rationale: The evidence supporting an association between traffic-related air pollution exposure and incident childhood asthma is inconsistent, and may depend on genetic factors.

Objectives: To identify gene-environment interaction effects on childhood asthma using genome-wide single nucleotide polymorphism (SNP) data and air pollution exposure. Identified loci were further analyzed at epigenetic and transcriptomic levels.

Methods: We used land use regression models to estimate individual air pollution exposure (represented by outdoor NO_2 levels) at the birth address and performed a genome-wide interaction study for doctor's diagnosis of asthma up to 8 years in three European birth cohorts (n=1,534) with look-up for interaction in two separate North American cohorts, CHS and CAPPS/SAGE (n=1,602 and 186 subjects, respectively). We assessed eQTL effects in human lung specimens and blood, as well as associations between air pollution exposure, methylation and transcriptomic patterns.

Measurements and Main results: In the European cohorts, 186 SNPs had an interaction p-value<$1x10^{-4}$ and look-up evaluation of these disclosed eight SNPs in four loci with interaction p<0.05 in the large CHS study, but not in CAPPS/SAGE. Three SNPs within *ADCY2* showed same direction of interaction effect, and were found to influence *ADCY2* gene expression in peripheral blood (p=$4.50x10^{-4}$). One other SNP with p<0.05 for interaction in CHS, rs686237,

strongly influenced *B4GALT5* expression in lung tissue ($p=1.18x10^{-17}$). Air pollution exposure was associated with differential *DLG2* methylation and expression.

Conclusion: Our results indicate that gene-environment interactions are important for asthma development and provide supportive evidence for interaction with air pollution for *ADCY2, B4GALT5* and *DLG2*.

Word count abstract: 250/250 words

Key words: genome-wide interaction study; methylation; gene expression; eQTL; children.

AJRCCM Articles in Press. Published on 30-November-2016 as 10.1164/rccm.201605-1026OC

Introduction

Asthma is the most common chronic disease among children.(1) Heredity is a well-known risk factor, exemplified by strong associations between chromosome 17q21 variants and childhood asthma,(2) but genetic factors cannot solely explain the increasing prevalence in the last decades. Exposure to traffic-related air pollution in early childhood (often indicated by the level of nitrogen dioxide (NO_2)) has been associated with asthma exacerbations (3) and reduced lung function in children,(4-7) but association with initial asthma development have been less consistent.(8-11)

The exact mechanisms by which air pollution may lead to asthma are incompletely understood. Oxidative stress and inflammation represent pathogenic pathways involved in asthma development.(3) Interactions between air pollution and allele variants in genes related to anti-oxidative stress systems, inflammation and innate immunity have been reported in relation to asthma incidence.(12, 13) Such gene-environment interactions may partially explain the inconsistencies between air pollution and asthma incidence. A limitation of these previous studies is that they have only included candidate genes and so far, no genome-wide attempt has been made.

We aimed to identify mechanisms of childhood asthma using genome-wide single nucleotide polymorphism (SNP) data and individual traffic-related air pollution exposure data, here expressed as exposure to NO_2 (see Figure E1 in the online data supplement). We present genome-wide interaction data from >1,500 asthmatic and non-asthmatic children from Three European birth cohorts in the discovery phase, followed by look-up in two independent North American cohorts consisting of almost 1,800 children. For each of the SNPs that were nominally significant for interaction in the largest look-up evaluation cohorts ($p<0.05$), we evaluated

expression quantitative trait locus (eQTL) effects in human lung specimens and traffic-related air pollution induced gene expression by genotype in peripheral blood cells along with effects of short- and long-term air pollution exposure on peripheral blood DNA methylation patterns. Some of the results of these studies have been previously reported in the form of an abstract.(14)

METHODS (1092/500 words)

Additional details are available in the online data supplement.

Study subjects

In the discovery phase, meta-analysis was performed based on GWIS results from three TAG (traffic pollution, asthma, genetics) consortium(13) cohorts including 454 asthmatic and 1,080 non-asthmatic children of European ancestry: BAMSE(6), Stockholm, Sweden (n asthmatic=235, n non-asthmatic=246), GINIplus(15) and LISAplus(16), Germany (n asthmatic=64, n non-asthmatic=661), and PIAMA(17), the Netherlands (n asthmatic=155, n non-asthmatic=173).Detailed cohort descriptions are provided in the online data supplement and elsewhere.(13)

The top discovery SNPs were further evaluated in two independent look-up datasets from North America consisting of 692 asthmatic and 1,096 non-asthmatic children in total: the birth cohorts CAPPS (Vancouver and Winnipeg, Canada)(18) and SAGE (Manitoba, Canada)(19), both of which include children with Caucasian ancestry, contributed 49 asthmatics and 137 non-asthmatics; the larger cohort Children's Health Study (CHS, California, USA)(20) which includes children of Non-Hispanic white ancestry, contributed 643 asthmatics and 959 non-asthmatics. All cohorts obtained ethical approval from their local review board.

AJRCCM Articles in Press. Published on 30-November-2016 as 10.1164/rccm.201605-1026OC

Exposure and outcome assessment

For the European birth cohorts, annual average of NO_2 exposure estimates at birth were derived using land use regression modeling (LUR). Site specific LUR models were developed and validated using the standardized European Study of Cohorts for Air pollution Effects (ESCAPE) project procedures (www.escapeproject.eu/manuals), as previously described in detail.(21) Using a similar methodology, LUR models of birth exposure were developed for CAPPS and SAGE.(22, 23) In CHS, NO_2 exposure was estimated based on the level in the child's community at baseline (mean age 8.8 years) obtained from central site monitors placed in each of the study communities.(20, 24) NO_2 was used as a proxy for traffic-related air pollution. Exposure data were entered as a continuous non-transformed variable and the risk estimates were reported per 10 $\mu g/m^3$ increase in NO_2 (Table E1).

Asthma definitions were based on parental reports of an ever doctor's diagnosis (BAMSE, GINI/LISA, PIAMA and CHS), clinical examinations by a pediatric allergist (CAPPS) or parental reports with confirmation of diagnoses by pediatric allergist (SAGE) (Table E2).

Genotyping and quality control

Genotyping, imputation procedure and quality control steps for each study are described in the online data supplement text and Table E1.

SNPxNO_2 interaction and asthma

As the primary model, logistic regression analyses for estimation of standard SNPxNO_2 interaction effects on asthma (multiplicative interaction model using HapMap2 imputed GWAS

data) was performed in each cohort separately. A genome-wide significant threshold of $p<7.2x10^{-8}$ for SNPxNO_2 interaction effects was applied.(25) Discovery meta-analysis of 2,082,301 overlapping SNPs was conducted using the statistical software METAL with fixed effect models with default METAL weights. . In addition to the primary SNPxNO_2 GWIS analysis, two other statistical methods to test for genome-wide interaction were used, with the same exposure, outcome and adjustment factors: a two-step approach, where in step one, the hypothesis of H0: $\beta_{SNP}=0$ was tested using NO_2 as outcome in a combined set of cases and controls. A subset of SNPs that exceeded a given significance threshold ($p<0.05$) for the test in step one was further analyzed in step two (in our study $N_{SNPs}=119,521$, equivalent to a genome-wide significance threshold of meta-analysis $p<4x10^{-7}$ after Bonferroni correction of 119,521 tests); testing the hypothesis that H0: $\beta_{SNP*NO2}=0$ (analyzing cases and controls; regular GxE interaction test).(26) The second method was the two degree of freedom (2 df) test that jointly test SNP main and SNPxNO_2 interaction effects. (27)

To avoid false negative findings, an arbitrary cut-off level for look-up of interacting SNPs was set at $p<1x10^{-4}$ (28) for our primary analysis in the discovery datasets (standard interaction model). Thus, SNPs with a combined interaction $p<1x10^{-4}$ in the discovery phase were selected for look-up evaluation of standard SNPxNO_2 interaction effects on asthma in the CAPPS/SAGE and CHS cohorts. Next, SNPs with $p<0.05$ for interaction in the larger CHS cohort (and annotated genes) were included in the functional genomics follow-up described below.

Gene expression analysis in lung tissue and peripheral blood cells

AJRCCM Articles in Press. Published on 30-November-2016 as 10.1164/rccm.201605-1026OC

Expression quantitative trait locus (eQTL) analyses were performed to evaluate if the SNPs significant in the look-up ($p<0.05$) were related to *cis*-acting lung tissue gene expression. Lung-tissue from 1,111 human subjects who underwent lung surgery at three academic sites, Laval University, University of British Columbia (UBC) and University of Groningen, have been previously analyzed.(29, 30) Linear regression models were used separately for each cohort adjusting for age, sex and smoking status. Meta-analysis was performed using inverse variance weighting. SNPs were considered an eQTL if they survived 5% B-H FDR correction for multiple testing of the number of gene probes tested for each SNP. The Genotype-Tissue Expression (GTEx) portal (http://www.gtexportal.org/home/) which provides tissue specific global gene expression data from genotyped donors was next used to evaluate whole blood eQTLs (n=338 samples), analyzing the same SNPs and genes as in the lung eQTL.(31) Furthermore, gene expression analyses (Affy HTA 2.0) were performed in peripheral blood cells from 263 16-year-olds in the BAMSE cohort as part of the MeDALL project.(32, 33) A look-up of GTEx identified eQTLs was performed in 173 BAMSE samples with GWAS data available using linear regression adjusting for age, sex and peripheral blood cell count. In addition, 250 BAMSE samples with exposure data available were used for linear regression association between NO_2 at birth / current NO_2 exposure at 16 years and expression levels of the genes annotated to significant look-up SNPs, with further stratification by genotype.

DNA methylation in relation to long- and short-term air pollution exposure

Methylation values for CpG sites within regions ±50 kb up and downstream of the identified genes were derived from Illumina 450K datasets and investigated for association with air pollution exposure. Methylation data from the BAMSE cohort at 8 years (n=460 with Illumina

450k data available) were investigated for association with long-term NO_2 exposure at birth using robust linear regression adjusting for age, sex, environmental tobacco smoke exposure during the first year of life, municipality at birth, ever doctor's diagnosis of asthma up to 8 years of age, celltype and batch (bisulfite treatment date).(33) The same CpG sites were also investigated for methylation quantitative trait locus (methQTL) effects to evaluate if the significant SNPs in the GxE look-up analyses were associated with methylation changes.

Short-term diesel exhaust exposure (DEP), as a model of particulate air pollution, was next investigated for association with DNA methylation difference in blood samples from sixteen 19- to 35-year-old non-smokers with asthma and/or airway hyper-responsiveness using linear mixed effects modeling to compare post-DEP vs. pre-DEP, and post-filtered air particles vs. pre-filtered air particles.(34) Adjustment was done using a 5% B-H FDR correction for multiple testing on the set of probes selected for the analysis.

RESULTS

Tables E1 and E2 in the online data supplement present the characteristics of the three European and two North American studies including NO_2 exposure assessment, genotyping and imputation procedures.

SNPxNO_2 interaction and asthma

In total, 1,534 children of European ancestry aged 7.4-11.3 years were included in the primary GWIS meta-analysis (454 asthmatics and 1,080 non-asthmatic controls). Figure E2 in the online

AJRCCM Articles in Press. Published on 30-November-2016 as 10.1164/rccm.201605-1026OC

data supplement shows the QQ-plot for the SNPxNO_2 interaction analysis on asthma (λ=1.03). The discovery meta-analysis provided no genome-wide significant hits at the genome-wide significant threshold of $p<7.2x10^{-8}$. The top SNPs for interaction effects (lowest $p=1.87x10^{-7}$) are located in chromosome 3p14.1 approximately 244 kilo-bases (kb) downstream of the membrane associated guanylate kinase, WW and PDZ domain containing 1 (*MAGI1)* gene (Figure 1 and Table E3, in the online data supplement). Next, we used an alternative two-step analytical approach suggested to increase power to detect gene-environment interactions.(26) Four SNPs reached genome-wide significance in this two-step model ($p<4x10^{-7}$, Table E4 in the online data supplement): rs7651862, rs11706125, rs11718057 and rs13066946 close to the *MAGI1* gene and these were also identified as top hit SNPs in the primary GWIS meta-analysis. As a third approach, we applied the 2 df test that jointly tests main SNP and SNPxNO_2 interaction effects.(27) No SNP reached genome-wide significance in this test (lowest p-value $1.08x10^{-6}$; Figure E3, Figure E4 and Table E5 in the online data supplement).

Look-up evaluation

We selected 186 interaction-effect SNPs with p-value$<1x10^{-4}$ from our primary model, the discovery GWIS meta-analysis for look-up in two different cohorts (Table E3 in the online data supplement). Of these 186 SNPs, 172 were available for look-up in the larger CHS imputed genome-wide SNP dataset (643 asthmatic children and 959 controls) and 8 SNPs showed nominal significant interaction ($p<0.05$) (Table 1 and Table E9 in the online data supplement). The SNP with the lowest p-value for interaction in CHS (rs686237, p=0.0016) is located on chromosome 20q13 in a region located 40 kb and 59 kb upstream of the genes UDP-Gal:betaGlcNAc beta 1,4- galactosyltransferase, polypeptide 5 (*B4GALT5)* and solute carrier

family 9, subfamily A (NHE8, cation proton antiporter 8), member 8 *(SLC9A8)*, respectively. Three SNPs (rs1057251, rs12455842, and rs12457919) are located downstream of, or within molybdenum cofactor sulfurase (*MOCOS),* on chromosome 18q12 and were in complete LD (r^2=1.0). These three SNPs were also among the top SNPs ($p<1x10^{-4}$) in the two-step interaction approach meta-analysis (Table E4 in the online data supplement). Three additional SNPs located within adenylate cyclase 2 (*ADCY2)* on chromosome 5p15.3 (rs4143882, rs727432, and rs6886921 with high LD, r^2=0.93-1.0) and one within discs, large homolog 2 (*DLG2)* on chromosome 11q14.1 (rs963146) reached nominal significance (p<0.05) (Table 1). The four SNPs close to the *MAGI1* gene and the eight SNPs with p<0.05 in CHS were also nominally significant in the 2 df test (p-value range $5.64x10^{-5}$-0.008, Table E6 in the online data supplement). In the smaller Canadian CAPPS and SAGE imputed genome-wide SNP dataset (49 asthmatic children and 137 controls), 122 SNPs were available for look-up evaluation. Two of the SNPs (rs3843891 on chromosome 4q31 and rs17265947 on chromosome 8q12.3) reached nominal significance (p<0.05) (Table E7 in the online data supplement), but none of the SNPs that were significant in CHS. The top SNPs close to*MAGI1* identified in the discovery GWIS meta-analysis and the two-step approach were not significant in CHS or CAPPS/SAGE. No overall significant main effects of the 8 SNPs on asthma were observed (Table E8 in the online data supplement). NO_2 exposure at birth (per 10μg/m^3 increase) was positively associated with asthma up to 8 years of age, albeit not statistically significant (meta-analysis adjusted OR 1.26 (0.61-2.58).

Direction of interaction effect and asthma risk

AJRCCM Articles in Press. Published on 30-November-2016 as 10.1164/rccm.201605-1026OC

SNPs that showed nominal significance in the larger CHS sample (all 8 SNPs in Table 1) were investigated for their direction and strength of effect in the association between NO_2 exposure and childhood asthma. Consistent directions of interaction effect between the discovery meta-analysis and CHS studies were identified for all three SNPs within *ADCY2* (Table 1) with increased risk of asthma associated with NO_2 exposure in carriers of the minor alleles. The stratified analyses did not show a consistent pattern of asthma risk for the other SNPs and the odds ratios for asthma across genotypes varied substantially between the datasets. Given the fact that the discovery datasets used exposure at birth and the main look-up study, CHS, used exposure at school-age, meta-analyses of the interaction effects were not meaningful since the odds ratios for asthma would represent different measures.

Gene expression analysis in lung tissue and peripheral blood

We performed eQTL analyses to evaluate if the 8 nominally significant SNPs from the look-up showed *cis*-acting eQTL associations in lung tissue (n=1,111). Rs686237 was identified as a highly significant cis-eQTL of *B4GALT5* (the C allele being associated with increased expression, $p=1.18x10^{-17}$, Figure 2, Table E10 in the online data supplement). In addition, rs12455842 was a significant *cis*-eQTL of *SLC39A6* (p=0.003, Table E10 in the online data supplement) in lung tissue. No other SNP showed significant *cis*-eQTL association in lung tissue after 5% FDR correction for multiple testing. GTEx eQTL analyses in whole blood confirmed that rs686237 was a significant *cis*-eQTL of *B4GALT5* ($p=4.00x10^{-4}$, Figure E5 in online supplement) but with opposite effect in that the C allele was associated with decreased expression. Rs6886921, rs727432 and rs4143882 were significant *cis*-eQTLs for *ADCY2* (lowest $p=4.50x10^{-4}$ for rs6886921 with the T allele being associated with decreased expression, Figure

E6 and Table E11 in the online data supplement). These blood eQTLs were however not statistically significant in the smaller BAMSE dataset (n=173).

Next, we explored NO_2 exposure association with gene expression in BAMSE (n=250). NO_2 exposure at birth was significantly influencing *ADCY2, DLG2* and *MOCOS* expression with increased expression levels in peripheral blood cells in relation to NO_2 (Table 2; similar associations seen also for exposure at 16 years, Table E12 in the online data supplement). For the top lung eQTL SNP, rs686237, an interacting SNPxNO_2 effect was detected for *B4GALT5* expression (interaction p-value=0.001, also FDR significant; Table 2) where the effect of NO_2 exposure at birth on gene expression differed depending on genotype status.

DNA methylation and air pollution exposure

Since air pollution exposure has been associated with differential DNA methylation patterns in peripheral blood cells,(35) we explored potential links between NO_2 exposure and methylation at the 278 CpG sites identified in a region ± 50 kb of the identified genes.

In the BAMSE cohort (n=460), NO_2 exposure at birth was significantly associated with 2.7% decreased methylation in CpG site cg02275784 within *DLG2* (per 10µg/m^3 NO_2 increase, p=1.21 x 10^{-4}). Methylation in other CpG sites was not associated with NO_2 exposure after 5% FDR correction for multiple testing (data not shown). Minor effects of DNA methylation changes were detected in the methQTL analysis with a level of methylation change up to 1% per allele (nominal p-values<0.05). None of the associations remained significant at the 5% FDR level (Table E13 in the online data supplement).

As a marker for short-term traffic-related air pollution exposure, 16 adult non-smoking asthmatics were exposed to two hours diesel (DE) exposure, at an average

AJRCCM Articles in Press. Published on 30-November-2016 as 10.1164/rccm.201605-1026OC

concentration of 300 $\mu g/m^3$, containing high levels of NO_2 (0.22 ppm).(34, 36) Difference in DNA methylation level was tested in blood samples pre- vs. post exposure. A total of 13 CpG sites were differentially methylated after 5% FDR correction for multiple testing (Table 3). Decreased methylation at eight CpG sites at *DLG2* locus was detected (lowest $p=4.64x10^{-5}$ for a 2% difference, cg26449294) and increased methylation was detected at two CpG sites close to transcription start sites (lowest $p=1.07x10^{-4}$ for a 4% difference, cg20275558) (Table 3). Decreased methylation was also identified at one *ADCY2* CpG site, and increased methylation was seen at one *MOCOS* CpG site.

DISCUSSION

We present a comprehensive GWIS with functional follow-up integrating genomics and environmental data that identified novel and previously identified loci for childhood asthma in relation to traffic-related air pollution exposure. Identified loci from the genome-wide SNP by NO_2 interaction approach, with significant look-up in 1,602 independent samples, were investigated for effects at genomic, epigenomic and transcriptomic levels. We provide supportive evidence for interaction with air pollution for the novel loci *B4GALT5* and the previously lung disease associated loci *ADCY2* (37, 38) and *DLG2*.(39)

The GWIS was used as a screening to detect genomic regions with a potential link to traffic-related air pollution exposure and childhood asthma. The SNP with the lowest p-value in the look-up evaluation, rs686237 on chromosome 20 was found to be a strong eQTL for expression of *B4GALT5* in the lung and was also identified as an eQTL for *B4GALT5* in whole blood. These results suggest a potential SNP-mediated effect of the association between NO_2 and

childhood asthma with a functional consequence as indicated by differential *B4GALT5* expression in blood depending on genotype.

The enzyme B4galt5 is involved in the biosynthesis of Lactosylceramide, which is a common precursor of glycosphingolipids.(40) Previous GWAS have identified a locus on chromosome 17q21, encompassing ORMDL sphingolipid biosynthesis regulator 3 (*ORMDL3*) and gasdermin B (*GSDMB*), to be strongly associated with childhood asthma.(2) Interestingly, the endoplasmic reticulum transmembrane protein ORMDL3 is involved in the regulation of eosinophil trafficking (41)

ADCY2 encodes a member of the family of adenylate cyclases, which are membrane-associated enzymes involved in G-protein coupled receptor signaling. Three SNPs in *ADCY2* showed statistical significance in the look-up evaluation of SNPxNO_2 interaction effects on asthma and they had all similar direction of effect between the discovery cohorts and the main look-up study, CHS. The three SNPs were also identified as eQTLs for *ADCY2* in whole blood. For the *ADCY2* eQTL rs6886921, the minor allele T was associated with decreased expression in blood, and CT/TT carriers had the highest risk of asthma associated with NO_2 exposure in both the discovery and CHS datasets. *ADCY2* was also differentially expressed in relation to air pollution exposure and decreased methylation levels were found in relation to short-term air pollution exposure. *ADCY2* SNPs have been previously associated to pulmonary function and chronic obstructive pulmonary disease (COPD).(37, 38, 42)

Using the complementary alternative two-step statistical approach for the genome-wide interaction analysis, genome-wide significance was reached in the discovery dataset for four SNPs located near the *MAGI1* locus. MAGI1 acts as a scaffolding protein, stabilizing and recruiting various molecules to the cell–cell contacts and is widely distributed at tight junctions

AJRCCM Articles in Press. Published on 30-November-2016 as 10.1164/rccm.201605-1026OC

in epithelial cells.(43) Involvement of the airway epithelium is of importance in asthma pathogenesis as disruption of barrier functions could potentially lead to air pollution-related adverse effects. The genome-wide significant SNPxNO_2 interaction results for *MAGI1* did however not show statistical significance for interaction in the look-up evaluation, and functional analyses were therefore not pursued. The 2 df test that jointly tests for main genetic and interaction effects is an attractive method in genome-wide interaction studies (27). It was primarily developed to detect main effects while fully taking the environmental exposure into account, and has been successfully used in large-scale lung function studies. (44) However, our analyses revealed no statistically significant hits at the genome-wide level, and limited power may have contributed to these results. The choice of method to detect interactions depends on study aims and availability of data and from our study, it is difficult to draw conclusions about any preferred model. The main focus in our study has been to perform functional interaction follow-up analyses on promising hits identified in the GWIS analyses, which we believe, is of crucial importance.

In the diesel exposure study on adults, methylation changes were most notable for CpG sites in the *DLG2* gene with reduced methylation levels at most sites. Analyses of long-term NO_2 exposure and DNA methylation profiles also indicated an association between air pollution exposure and *DLG2* methylation changes. We did not identify any significant association between the top *DLG2* SNP rs963146 and *DLG2* methylation indicating that the difference in *DLG2* methylation levels associated with air pollution is not SNP mediated. NO_2 exposure was associated with higher expression levels of *DLG2* in blood cells (Table 2), which provides further evidence that exposure may induce functional changes related to this gene. DLG2 (and MAGI1) belongs to the membrane-associated guanylate kinase (MAGUK) family.(45)

Disruption of *Drosophila melanogaster* DLG results in acute disorganization of epithelial structure with disruption of intercellular junction formation.(45) *DLG2* has recently been associated with COPD.(39)

Three *MOCOS* SNPs were nominally significant in the CHS study, but we did not find convincing data in our functional analyses to support gene-environment interactions of importance.

FANTOM5(46) and the Human Protein Atlas (HPA)(47) results show that the identified genes are expressed at mRNA and protein levels in tissues relevant for the asthmatic disease, although B4GALT5 could not be evaluated for protein expression in HPA (see online data supplement for additional details).

This study included all available datasets that we are aware of with the required childhood phenotype, exposure and genetic data needed for interaction analyses. Nevertheless, it would have been preferable to have larger sample sizes for GxE analyses and functional analyses to decrease the likelihood of both type I and type II errors, and inclusion of non-White populations would have increased the generalizability of our results. We acknowledge that none of the identified SNPs was actually genome-wide significant in the discovery dataset and at the same time, significant in the look-up datasets. Low statistical power is common in studies using GWIS data, and previous GWIS efforts to detect gene-environment interaction effects for asthma and lung function indicate that new loci are challenging to discover.(44, 48, 49)

For all cohorts, exposures were based on modeled outdoor concentrations of NO_2 (a surrogate for traffic-related pollution) at the home and school addresses, but personal exposure to different pollution components including indoor exposures were not considered. NO_2 level is a good indicator for local air pollution, mainly from motor vehicles, and is highly correlated with

AJRCCM Articles in Press. Published on 30-November-2016 as 10.1164/rccm.201605-1026OC

other components of motor vehicle emissions, such as exhaust particles.(21) However, we observed quite heterogeneous interaction effects in the discovery and look-up datasets, and only *ADCY2* SNPs showed similar directions of effect in the discovery and main look-up study. Differences in the levels or constituents of air pollutants, co-exposures and unmeasured confounding factors between the North American and European cohorts could possibly explain the observed results. We also acknowledge that we used a rather liberal and unspecific definition of asthma (similar to the GABRIEL GWAS(2)), and the maximum age of asthma definition in CHS was up to three years older compared to the other cohorts, which may have contributed to heterogeneous effects.(50) Given these differences, we did not perform meta-analysis of the interaction betas but presented interaction betas and p-values for each dataset.

Previous gene-environment interaction analyses using candidate gene approaches have suggested genes related to anti-oxidative stress systems, inflammation and innate immunity, such as *GSTP1*, *TNF* and *TLR2/4*, as important effect modifiers.(12, 13) These genes were not among the top hits in our GWIS, but this does not exclude true interaction effects for key SNPs as previously reported.

A key strength of our study is the extensive functional follow-up, and we provide data for asthma that indicate the involvement of identified genes at the genomic, epigenomic and transcriptomic levels in both lung tissue and peripheral blood cells in relation to air pollution exposure. These results are unlikely to be biased due to ethnic differences in our study populations because all data was based on an ethnically homogenous (European or Non-Hispanic white ancestry) population. In all steps of our study, we corrected for multiple testing to minimize false positive findings.

Our gene-environment interaction analysis using genome-wide data and multiple functional DNA methylation and gene expression analyses provides promising results for further understanding of the pathogenesis of childhood asthma. Our results support the notion that gene-environment interactions are important for asthma development, and that functional genomics analyses in conjunction with detailed environmental exposures provide valuable insight about pathophysiologic mechanisms.

AJRCCM Articles in Press. Published on 30-November-2016 as 10.1164/rccm.201605-1026OC

ACKNOWLEDGEMENTS

We would like thank all the families for their participation in the BAMSE study. In addition, we would like to thank Eva Hallner, André Lauber and Sara Nilsson at the BAMSE office for invaluable support. We would also like to thank all the families for their participation in the GINIplus and LISAplus studies. Furthermore, we thank all members of the GINIplus and LISAplus Study Groups for their excellent work. The LISAplus Study Group consists of the following: Helmholtz Zentrum Muenchen - German Research Center for Environment and Health, Institute of Epidemiology I, Neuherberg (Heinrich J, Wichmann HE, Sausenthaler S, Chen C-M); University of Leipzig, Department of Pediatrics (Borte M), Department of Environmental Medicine and Hygiene (Herbarth O); Department of Pediatrics, Marien-Hospital, Wesel (von Berg A); Bad Honnef (Schaaf B); UFZ-Centre for Environmental Research Leipzig-Halle, Department of Environmental Immunology (Lehmann I); IUF – Leibniz Research Institute for Environmental Medicine, Düsseldorf (Krämer U); Department of Pediatrics, Technical University, Munich (Bauer CP, Hoffman U). The GINIplus Study Group consists of the following: Helmholtz Zentrum Muenchen - German Research Center for Environmental Health, Institute of Epidemiology I, Munich (Heinrich J, Wichmann HE, Sausenthaler S, Chen C-M, Thiering E, Tiesler C, Standl M, Schnappinger M, Rzehak P); Department of Pediatrics, Marien-Hospital, Wesel (Berdel D, von Berg A, Beckmann C, Groß I); Department of Pediatrics, Ludwig Maximilians University, Munich (Koletzko S, Reinhardt D, Krauss-Etschmann S); Department of Pediatrics, Technical University, Munich (Bauer CP, Brockow I, Grübl A, Hoffmann U); IUF – Leibniz Research Institute for Environmental Medicine, Düsseldorf (Krämer U, Link E, Cramer C); Centre for Allergy and Environment, Technical University, Munich (Behrendt H). The PIAMA birth cohort study is a collaboration of the Institute for Risk

Assessment Sciences, University Utrecht (B. Brunekreef), Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht (H.A. Smit), Centre for Prevention and Health Services Research, National Institute for Public Health and the Environment, Bilthoven (A.H. Wijga), Department of Pediatrics, Division of Respiratory Medicine, Erasmus MC -Sophia, Rotterdam (J.C. de Jongste), Pulmonology (D.S. Postma) and Pediatric Pulmonology and Pediatric Allergology (G.H. Koppelman) of the University Medical Center Groningen and the Department of Immunopathology, Sanquin Research, Amsterdam (R.C. Aalberse), The Netherlands. The study team gratefully acknowledges the participants in the PIAMA birth cohort study, and all coworkers who helped conducting the medical examinations, field work and data management. We acknowledge Denise Daley and the AllerGen Genetics team for assistance with CAPPS and SAGE data management and transfer.

AJRCCM Articles in Press. Published on 30-November-2016 as 10.1164/rccm.201605-1026OC

REREFENCES

1. Asher MI, Montefort S, Bjorksten B, Lai CK, Strachan DP, Weiland SK, Williams H. Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: ISAAC Phases One and Three repeat multicountry cross-sectional surveys. *Lancet* 2006; 368: 733-743.

2. Moffatt MF, Gut IG, Demenais F, Strachan DP, Bouzigon E, Heath S, von Mutius E, Farrall M, Lathrop M, Cookson WO, consortium. G. A large-scale, consortium-based genomewide association study of asthma. *The New England journal of medicine* 2010; 363: 1211-1221.

3. Carlsten C, Melen E. Air pollution, genetics, and allergy: an update. *Curr Opin Allergy Clin Immunol* 2012; 12: 455-460.

4. Gauderman WJ, Vora H, McConnell R, Berhane K, Gilliland F, Thomas D, Lurmann F, Avol E, Kunzli N, Jerrett M, Peters J. Effect of exposure to traffic on lung development from 10 to 18 years of age: a cohort study. *Lancet* 2007; 369: 571-577.

5. Gehring U, Gruzieva O, Agius RM, Beelen R, Custovic A, Cyrys J, Eeftens M, Flexeder C, Fuertes E, Heinrich J, Hoffmann B, de Jongste JC, Kerkhof M, Klumper C, Korek M, Molter A, Schultz ES, Simpson A, Sugiri D, Svartengren M, von Berg A, Wijga AH, Pershagen G, Brunekreef B. Air pollution exposure and lung function in children: the ESCAPE project. *Environmental health perspectives* 2013; 121: 1357-1364.

6. Schultz ES, Hallberg J, Bellander T, Bergstrom A, Bottai M, Chiesa F, Gustafsson PM, Gruzieva O, Thunqvist P, Pershagen G, Melen E. Early-Life Exposure to Traffic-related Air Pollution and Lung Function in Adolescence. *Am J Respir Crit Care Med* 2016; 193: 171-177.

7. Rice MB, Rifas-Shiman SL, Litonjua AA, Oken E, Gillman MW, Kloog I, Luttmann-Gibson H, Zanobetti A, Coull BA, Schwartz J, Koutrakis P, Mittleman MA, Gold DR. Lifetime Exposure to Ambient

Pollution and Lung Function in Children. *American journal of respiratory and critical care medicine* 2016; 193: 881-888.

8. Gruzieva O, Bergstrom A, Hulchiy O, Kull I, Lind T, Melen E, Moskalenko V, Pershagen G, Bellander T. Exposure to air pollution from traffic and childhood asthma until 12 years of age. *Epidemiology* 2013; 24: 54-61.

9. Gehring U, Wijga AH, Hoek G, Bellander T, Berdel D, Brüske I, Fuertes E, Gruzieva O, Heinrich J, Hoffmann B, de Jongste JC, Klümper C, Koppelman GH, Korek M, Krämer U, Maier D, Melén E, Pershagen G, Postma DS, Standl M, von Berg A, Anto JM, Bousquet J, Keil T, Smit HA, Brunekreef B. Exposure to air pollution and development of asthma and rhinoconjunctivitis throughout childhood and adolescence: a population-based birth cohort study. *The Lancet Respiratory Medicine* 2015; 3: 933-942.

10. Molter A, Simpson A, Berdel D, Brunekreef B, Custovic A, Cyrys J, de Jongste J, de Vocht F, Fuertes E, Gehring U, Gruzieva O, Heinrich J, Hoek G, Hoffmann B, Klumper C, Korek M, Kuhlbusch TA, Lindley S, Postma D, Tischer C, Wijga A, Pershagen G, Agius R. A multicentre study of air pollution exposure and childhood asthma prevalence: the ESCAPE project. *The European respiratory journal* 2015; 45: 610-624.

11. Bharadwaj P, Graff Zivin J, Mullins JT, Neidell M. Early Life Exposure to the Great Smog of 1952 and the Development of Asthma. *Am J Respir Crit Care Med* 2016.

12. Kerkhof M, Postma DS, Brunekreef B, Reijmerink NE, Wijga AH, de Jongste JC, Gehring U, Koppelman GH. Toll-like receptor 2 and 4 genes influence susceptibility to adverse effects of traffic-related air pollution on childhood asthma. *Thorax* 2010; 65: 690-697.

13. MacIntyre EA, Brauer M, Melen E, Bauer CP, Bauer M, Berdel D, Bergstrom A, Brunekreef B, Chan-Yeung M, Klumper C, Fuertes E, Gehring U, Gref A, Heinrich J, Herbarth O, Kerkhof M, Koppelman GH, Kozyrskyj AL, Pershagen G, Postma DS, Thiering E, Tiesler CM, Carlsten C, Group

AJRCCM Articles in Press. Published on 30-November-2016 as 10.1164/rccm.201605-1026OC

TAGS. GSTP1 and TNF Gene variants and associations between air pollution and incident childhood asthma: the traffic, asthma and genetics (TAG) study. *Environmental health perspectives* 2014; 122: 418-424.

14. Gref A, Merid S, Gruzieva O, Ballereau S, Becker A, Bellander T, Bergström A, Bossé Y, Bottai M, Chan-Yeung M, Fuertes E, Ierodakoniou D, Jiang R, Kobor M, Korek M, Kozyrskyj A, Kumar A, Lemonnier N, MacIntyre E, Nickle D, Obeidat M, Pellet J, Standl M, Sääf A, Söderhäll C, Tiesler C, van den Berge M, Vonk J, Vora H, Xu C, Antó J, Auffray C, Brauer B, Bousquet J, Brunekreef B, Gauderman W, Heinrich J, Kere J, Koppelman G, Postma D, Carlsten C, Pershagen G, Melén E. An integrative genomics approach identifies new asthma pathways related to air pollution. *The European respiratory journal* 2015; 46.
15. Berg A, Kramer U, Link E, Bollrath C, Heinrich J, Brockow I, Koletzko S, Grubl A, Filipiak-Pittroff B, Wichmann HE, Bauer CP, Reinhardt D, Berdel D, group GIs. Impact of early feeding on childhood eczema: development after nutritional intervention compared with the natural course - the GINIplus study up to the age of 6 years. *Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology* 2010; 40: 627-636.
16. Heinrich J, Bolte G, Holscher B, Douwes J, Lehmann I, Fahlbusch B, Bischof W, Weiss M, Borte M, Wichmann HE, Group LS. Allergens and endotoxin on mothers' mattresses and total immunoglobulin E in cord blood of neonates. *The European respiratory journal* 2002; 20: 617-623.
17. Wijga AH, Kerkhof M, Gehring U, de Jongste JC, Postma DS, Aalberse RC, Wolse AP, Koppelman GH, van Rossem L, Oldenwening M, Brunekreef B, Smit HA. Cohort profile: the prevention and incidence of asthma and mite allergy (PIAMA) birth cohort. *International journal of epidemiology* 2014; 43: 527-535.

18. Carlsten C, Dybuncio A, Becker A, Chan-Yeung M, Brauer M. Traffic-related air pollution and incident asthma in a high-risk birth cohort. *Occupational and environmental medicine* 2011; 68: 291-295.

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

20. Peters JM, Avol E, Navidi W, London SJ, Gauderman WJ, Lurmann F, Linn WS, Margolis H, Rappaport E, Gong H, Thomas DC. A study of twelve Southern California communities with differing levels and types of air pollution. I. Prevalence of respiratory morbidity. *Am J Respir Crit Care Med* 1999; 159: 760-767.

21. Beelen R, Hoek G, Vienneau D, Eeftens M, Dimakopoulou K, Pedeli X, Tsai MY, Kunzli N, Schikowski T, Marcon A, Eriksen KT, Raaschou-Nielsen O, Stephanou E, Patelarou E, Lanki T, Yli-Toumi T, Declercq C, Falq G, Stempfelet M, Birk M, Cyrys J, von Klot S, Nador G, Varro MJ, Dedele A, Grazuleviciene R, Molter A, Lindley S, Madsen C, Cesaroni G, Ranzi A, Badaloni C, Hoffmann B, Nonnemacher M, Kraemer U, Kuhlbusch T, Cirach M, de Nazelle A, Nieuwenhuijsen M, Bellander T, Korek M, Olsson D, Stromgren M, Dons E, Jerrett M, Fischer P, Wang M, Brunekreef B, de Hoogh K. Development of NO2 and NOx land use regression models for estimating air pollution exposure in 36 study areas in Europe - The ESCAPE project. *Atmos Environ* 2013; 72: 10-23.

22. Henderson SB, Beckerman B, Jerrett M, Brauer M. Application of land use regression to estimate long-term concentrations of traffic-related nitrogen oxides and fine particulate matter. *Environmental Science & Technology* 2007; 41: 2422-2428.

23. Allen RW, Amram O, Wheeler AJ, Brauer M. The transferability of NO and NO2 land use regression models between cities and pollutants. *Atmos Environ* 2011; 45: 369-378.

24. McConnell R, Berhane K, Gilliland F, Molitor J, Thomas D, Lurmann F, Avol E, Gauderman WJ, Peters JM. Prospective study of air pollution and bronchitic symptoms in children with asthma. *Am J Respir Crit Care Med* 2003; 168: 790-797.

AJRCCM Articles in Press. Published on 30-November-2016 as 10.1164/rccm.201605-1026OC

25. Dudbridge F, Gusnanto A. Estimation of significance thresholds for genomewide association scans. *Genet Epidemiol* 2008; 32: 227-234.

26. Murcray CE, Lewinger JP, Gauderman WJ. Gene-environment interaction in genome-wide association studies. *Am J Epidemiol* 2009; 169: 219-226.

27. Kraft P, Yen YC, Stram DO, Morrison J, Gauderman WJ. Exploiting gene-environment interaction to detect genetic associations. *Hum Hered* 2007; 63: 111-119.

28. Wu K, Gamazon ER, Im HK, Geeleher P, White SR, Solway J, Clemmer GL, Weiss ST, Tantisira KG, Cox NJ, Ratain MJ, Huang RS. Genome-wide interrogation of longitudinal FEV1 in children with asthma. *Am J Respir Crit Care Med* 2014; 190: 619-627.

29. Hao K, Bosse Y, Nickle DC, Pare PD, Postma DS, Laviolette M, Sandford A, Hackett TL, Daley D, Hogg JC, Elliott WM, Couture C, Lamontagne M, Brandsma CA, van den Berge M, Koppelman G, Reicin AS, Nicholson DW, Malkov V, Derry JM, Suver C, Tsou JA, Kulkarni A, Zhang C, Vessey R, Opiteck GJ, Curtis SP, Timens W, Sin DD. Lung eQTLs to help reveal the molecular underpinnings of asthma. *Plos Genet* 2012; 8: e1003029.

30. Lamontagne M, Timens W, Hao K, Bosse Y, Laviolette M, Steiling K, Campbell JD, Couture C, Conti M, Sherwood K, Hogg JC, Brandsma CA, van den Berge M, Sandford A, Lam S, Lenburg ME, Spira A, Pare PD, Nickle D, Sin DD, Postma DS. Genetic regulation of gene expression in the lung identifies CST3 and CD22 as potential causal genes for airflow obstruction. *Thorax* 2014; 69: 997-1004.

31. GTEx Consortium. The Genotype-Tissue Expression (GTEx) project. *Nat Genet* 2013; 45: 580-5.

32. Bousquet J, Anto JM, Akdis M, Auffray C, Keil T, Momas I, Postma D, Valenta R, Wickman M, Cambon-Thomsen A, Haahtela T, Lambrecht BN, Lodrup-Carlsen K, Koppelman GH, Sunyer J, Zuberbier T, Annesi-Maesano I, Arno A, Bindslev-Jensen C, De Carlo G, Forastiere F, Heinrich J, Kowalski ML, Maier D, Melen E, Palkonen S, Smit HA, Standl M, Wright J, Arsanoj A, Benet M,

Balardini N, Garcia-Aymerich J, Gehring U, Guerra S, Hohman C, Kull I, Lupinek C, Pinart M, Skrindo I, Westman M, Smagghe D, Akdis C, Albang R, Anastasova V, Anderson N, Bachert C, Ballereau S, Ballester F, Basagana X, Bedbrook A, Bergstrom A, von Berg A, Brunekreef B, Burte E, Carlsen KH, Chatzi L, Coquet JM, Curin M, Demoly P, Eller E, Fantini MP, Gerhard B, Hammad H, von Hertzen L, Hovland V, Jacquemin B, Just J, Keller T, Kerkhof M, Kiss R, Kogevinas M, Koletzko S, Lau S, Lehmann I, Lemonnier N, McEachan R, Makela M, Mestres J, Minina E, Mowinckel P, Nadif R, Nawijn M, Oddie S, Pellet J, Pin I, Porta D, Ranciere F, Rial-Sebbag A, Saes Y, Schuijs MJ, Siroux V, Tischer CG, Torrent M, Varraso R, De Vocht J, Wenger K, Wieser S, Xu C. Paving the way of systems biology and precision medicine in allergic diseases: The MeDALL success story. *Allergy* 2016.

33. Gruzieva O, Xu CJ, Breton CV, Annesi-Maesano I, Anto JM, Auffray C, Ballereau S, Bellander T, Bousquet J, Bustamante M, Charles MA, de Kluizenaar Y, den Dekker HT, Duijts L, Felix JF, Gehring U, Guxens M, Jaddoe VV, Jankipersadsing SA, Merid SK, Kere J, Kumar A, Lemonnier N, Lepeule J, Nystad W, Page CM, Panasevich S, Postma D, Slama R, Sunyer J, Soderhall C, Yao J, London SJ, Pershagen G, Koppelman GH, Melen E. Epigenome-Wide Meta-Analysis of Methylation in Children Related to Prenatal NO2 Air Pollution Exposure. *Environ Health Perspect* 2016.

34. Jiang R, Jones MJ, Sava F, Kobor MS, Carlsten C. Short-term diesel exhaust inhalation in a controlled human crossover study is associated with changes in DNA methylation of circulating mononuclear cells in asthmatics. *Part Fibre Toxicol* 2014; 11: 71.

35. Gruzieva O, Merid SK, Melén E. An update on epigenetics and childhood respiratory diseases. *Paediatric respiratory reviews* 2014; 15: 348-354.

AJRCCM Articles in Press. Published on 30-November-2016 as 10.1164/rccm.201605-1026OC

36. Birger N, Gould T, Stewart J, Miller MR, Larson T, Carlsten C. The Air Pollution Exposure Laboratory (APEL) for controlled human exposure to diesel exhaust and other inhalants: characterization and comparison to existing facilities. *Inhal Toxicol* 2011; 23: 219-225.

37. Hancock DB, Eijgelsheim M, Wilk JB, Gharib SA, Loehr LR, Marciante KD, Franceschini N, van Durme YM, Chen TH, Barr RG, Schabath MB, Couper DJ, Brusselle GG, Psaty BM, van Duijn CM, Rotter JI, Uitterlinden AG, Hofman A, Punjabi NM, Rivadeneira F, Morrison AC, Enright PL, North KE, Heckbert SR, Lumley T, Stricker BH, O'Connor GT, London SJ. Meta-analyses of genome-wide association studies identify multiple loci associated with pulmonary function. *Nat Genet* 2010; 42: 45-52.

38. Castaldi PJ, Cho MH, Litonjua AA, Bakke P, Gulsvik A, Lomas DA, Anderson W, Beaty TH, Hokanson JE, Crapo JD, Laird N, Silverman EK. The association of genome-wide significant spirometric loci with chronic obstructive pulmonary disease susceptibility. *Am J Respir Cell Mol Biol* 2011; 45: 1147-1153.

39. Chen W, Brehm JM, Manichaikul A, Cho MH, Boutaoui N, Yan Q, Burkart KM, Enright PL, Rotter JI, Petersen H, Leng S, Obeidat M, Bosse Y, Brandsma CA, Hao K, Rich SS, Powell R, Avila L, Soto-Quiros M, Silverman EK, Tesfaigzi Y, Barr RG, Celedon JC. A Genome-Wide Association Study of Chronic Obstructive Pulmonary Disease in Hispanics. *Ann Am Thorac Soc* 2015.

40. Tokuda N, Numata S, Li X, Nomura T, Takizawa M, Kondo Y, Yamashita Y, Hashimoto N, Kiyono T, Urano T, Furukawa K. beta4GalT6 is involved in the synthesis of lactosylceramide with less intensity than beta4GalT5. *Glycobiology* 2013; 23: 1175-1183.

41. Ha SG, Ge XN, Bahaie NS, Kang BN, Rao A, Rao SP, Sriramarao P. ORMDL3 promotes eosinophil trafficking and activation via regulation of integrins and CD48. *Nat Commun* 2013; 4: 2479.

42. Panasevich S, Melen E, Hallberg J, Bergstrom A, Svartengren M, Pershagen G, Nyberg F. Investigation of novel genes for lung function in children and their interaction with tobacco smoke exposure: a preliminary report. *Acta Paediatr* 2013; 102: 498-503.

43. Facciuto F, Cavatorta AL, Valdano MB, Marziali F, Gardiol D. Differential expression of PDZ domain-containing proteins in human diseases - challenging topics and novel issues. *FEBS J* 2012; 279: 3538-3548.

44. Hancock DB, Soler Artigas M, Gharib SA, Henry A, Manichaikul A, Ramasamy A, Loth DW, Imboden M, Koch B, McArdle WL, Smith AV, Smolonska J, Sood A, Tang W, Wilk JB, Zhai G, Zhao JH, Aschard H, Burkart KM, Curjuric I, Eijgelsheim M, Elliott P, Gu X, Harris TB, Janson C, Homuth G, Hysi PG, Liu JZ, Loehr LR, Lohman K, Loos RJ, Manning AK, Marciante KD, Obeidat M, Postma DS, Aldrich MC, Brusselle GG, Chen TH, Eiriksdottir G, Franceschini N, Heinrich J, Rotter JI, Wijmenga C, Williams OD, Bentley AR, Hofman A, Laurie CC, Lumley T, Morrison AC, Joubert BR, Rivadeneira F, Couper DJ, Kritchevsky SB, Liu Y, Wjst M, Wain LV, Vonk JM, Uitterlinden AG, Rochat T, Rich SS, Psaty BM, O'Connor GT, North KE, Mirel DB, Meibohm B, Launer LJ, Khaw KT, Hartikainen AL, Hammond CJ, Glaser S, Marchini J, Kraft P, Wareham NJ, Volzke H, Stricker BH, Spector TD, Probst-Hensch NM, Jarvis D, Jarvelin MR, Heckbert SR, Gudnason V, Boezen HM, Barr RG, Cassano PA, Strachan DP, Fornage M, Hall IP, Dupuis J, Tobin MD, London SJ. Genome-wide joint meta-analysis of SNP and SNP-by-smoking interaction identifies novel loci for pulmonary function. *PLoS Genet* 2012; 8: e1003098.

45. Roberts S, Delury C, Marsh E. The PDZ protein discs-large (DLG): the 'Jekyll and Hyde' of the epithelial polarity proteins. *FEBS J* 2012; 279: 3549-3558.

46. Severin J, Lizio M, Harshbarger J, Kawaji H, Daub CO, Hayashizaki Y, Bertin N, Forrest AR. Interactive visualization and analysis of large-scale sequencing datasets using ZENBU. *Nat Biotechnol* 2014; 32: 217-219.

AJRCCM Articles in Press. Published on 30-November-2016 as 10.1164/rccm.201605-1026OC

47. Uhlen M, Oksvold P, Fagerberg L, Lundberg E, Jonasson K, Forsberg M, Zwahlen M, Kampf C, Wester K, Hober S, Wernerus H, Bjorling L, Ponten F. Towards a knowledge-based Human Protein Atlas. *Nat Biotechnol* 2010; 28: 1248-1250.

48. Melen E, Bottai M. On lung function and interactions using genome-wide data. *Plos Genet* 2012; 8: e1003174.

49. Scholtens S, Postma DS, Moffatt MF, Panasevich S, Granell R, Henderson AJ, Melen E, Nyberg F, Pershagen G, Jarvis D, Ramasamy A, Wjst M, Svanes C, Bouzigon E, Demenais F, Kauffmann F, Siroux V, von Mutius E, Ege MJ, Braun-Fahrlander C, Genuneit J, Brunekreef B, Smit HA, Wijga AH, Kerkhof M, Curjuric I, Imboden M, Thun GA, Probst-Hensch N, Freidin MB, Bragina E, Deev IA, Puzyrev VP, Daley D, Park J, Becker A, Chan-Yeung M, Kozyrskyj AL, Pare P, Marenholz I, Lau S, Keil T, Lee YA, Kabesch M, Wijmenga C, Franke L, Nolte IM, Vonk J, Kumar A, Farrall M, Cookson WO, Strachan DP, Koppelman GH, Boezen HM. Novel childhood asthma genes interact with in utero and early-life tobacco smoke exposure. *J Allergy Clin Immunol* 2014; 133: 885-888.

50. Sbihi H, Koehoorn M, Tamburic L, Brauer M. Asthma Trajectories in a Population-based Birth Cohort: Impacts of Air Pollution and Greenness. *Am J Respir Crit Care Med* 2016.

Figure legends

Figure 1. Manhattan plot for the discovery genome-wide interaction meta-analysis of the association between SNPxNO_2 and asthma. The horizontal red line indicates the genome-wide significance threshold when using the two-step interaction approach ($p<4x10^{-7}$). The horizontal blue line indicates the threshold for SNPs selected for look-up ($p<1x10^{-4}$, n=186). The locus, near *MAGI1* on chromosome 3p14.1, which reached genome-wide significance when using the two-step interaction approach is marked in green (rs7651862, rs11706125, rs11718057, rs13066946).

Figure 2. Gene expression levels of the *B4GALT5* gene in lung tissues according to genotyping groups for SNP rs686237 (using an additive model). The left, middle, and right panels are results from Laval (n=397, $p=3.86x10^{-4}$), UBC (n=281, p=0.0043) and Groningen (n=329, $p=8.92x10^{-4}$), respectively, with a meta-analysis p-value of $1.18x10^{-17}$. Expression is presented for probeset 100313047_TGI_at. The y-axis represents gene expression levels in the lung. The x-axis represents the three genotyping groups for SNP rs686237 (build 37 position 48,370,734) with the number of subjects in parenthesis. The right y-axis presents the percent variance in gene expression levels explained by the genotype.

AJRCCM Articles in Press. Published on 30-November-2016 as 10.1164/rccm.201605-1026OC

Table 1: SNPs from the genome wide interaction meta-analysis of the association between SNPxNO_2 interaction and asthma that were statistically significant in the look-up evaluation

				Discovery GWIS meta-analysis		**Look-up**			
				BAMSE, GINI/LISA, PIAMA		**CHS**		**CAPPS/SAGE**	
				n=1,534		**n=1,602**		**n=186**	
Chr	**SNP**	**MAF**	**Nearest gene**	**Interaction p-value***	**Stratification by genotype†: OR (95%CI)**	**Interaction p-value‡**	**Stratification by genotype†: OR (95%CI)**	**Interaction p-value‡**	**Stratification by genotype†: OR (95%CI)**
20	rs686237	0.32	*B4GALT5, SLC9A8*	$5.43x10^{-5}$	CC: 0.77 (0.48-1.24) AC/AA: 1.69 (1.08-2.64)	0.0016	CC: 1.21 (1.04-1.41) AC/AA: 0.89 (0.78-1.01)	NA	NA
18	rs1057251	0.10	*MOCOS*	$6.18x10^{-5}$	TT: 1.68 (0.85-3.29) CT/CC: 0.50 (0.22-1.15)	0.0094	TT: 0.95 (0.85-1.06) CT/CC: 1.30 (1.03-1.62)	0.58	TT: 2.59 (1.01-6.66) CT/CC: 2.02 (0.06-66.02)
18	rs12455842	0.10	*MOCOS*	$6.10x10^{-5}$	TT: 1.70 (0.86-3.39) CT/CC: 0.48 (0.21-1.10)	0.010	TT: 0.95 (0.85-1.06) CT/CC: 1.30 (1.03-1.62)	0.55	TT: 2.59 (1.01-6.66) CT/CC: 2.02 (0.06-66.02)
5	rs4143882	0.33	*ADCY2*	$4.75x10^{-5}$	GG: 0.81 (0.33-1.99) AG/AA: 1.61 (1.04-2.51)	0.015	GG: 0.88 (0.76-1.02) AG/AA: 1.13 (0.98-1.29)	0.26	GG: 4.90 (1.25-19.24) AG/AA: 1.04 (0.26-4.24)
5	rs727432	0.32	*ADCY2*	$6.67x10^{-5}$	GG:0.81 (0.33-1.99) GT/TT: 1.61 (1.04-2.51)	0.016	GG: 0.88 (0.76-1.02) GT/TT: 1.13 (0.98-1.29)	0.27	GG: 4.90 (1.25-19.24) GT/TT: 1.04 (0.26-4.24)
5	rs6886921	0.34	*ADCY2*	$7.03x10^{-6}$	CC:0.76 (0.29-1.99) CT/TT: 1.71 (1.11-2.66)	0.016	CC: 0.88 (0.76-1.02) CT/TT: 1.12 (0.98-1.27)	NA	NA
18	rs12457919	0.10	*MOCOS, FHOD3*	$5.52x10^{-5}$	AA: 1.68 (0.85-3.29) AC/CC: 0.39 (0.09-1.75)	0.012	AA: 0.95 (0.85-1.06) AC/CC: 1.30 (1.03-1.62)	NA	NA
11	rs963146	0.21	*DLG2*	$8.61x10^{-5}$	AA: 1.56 (1.04-2.33)	0.034	AA: 0.93 (0.83-1.06)	0.62	AA: 3.02 (0.84-10.87)

AJRCCM Articles in Press. Published on 30-November-2016 as 10.1164/rccm.201605-1026OC

	AG/GG: 0.67 (0.21-2.18)	AG/GG: 1.12 (0.96-1.32)	AG/GG: 2.75 (0.70-10.79)

Shown are SNPs that were nominally significant in CHS (p-value<0.05), ordered by CHS interaction p-value. All p-values given are two-sided. Chr, chromosome; MAF, minor allele frequency, according to BAMSE; OR, odds ratio for asthma associated with exposure to traffic-NO_2 for different genotypes; NA, not available. *Genome-wide significance threshold, $p<7.2x10^{-8}$. †Stratification by genotype using dominant model. ‡Significance threshold for look-up evaluation, p<0.05.

AJRCCM Articles in Press. Published on 30-November-2016 as 10.1164/rccm.201605-1026OC

Table 2. Association between NO_2 exposure levels at birth and peripheral blood gene expression levels at 16 years of age in BAMSE (n=250*).

Chr	Gene	probe	Associated SNP	Genotype	Coef	p-value	Interaction p-value
5	*ADCY2*	TC05000054.hg.1		All, n=250	0.03	**0.05**	0.85
			rs6886921	CC n=72	0.04	0.17	
				TC n=83	0.04	0.17	
				TT n=18	-0.07	0.19	
5	*ADCY2*	TC05000055.hg.1		All, n=250	0.04	0.09	0.17
			rs6886921	CC n=72	0.08	0.07	
				TC n=83	-0.05	0.53	
				TT n=18	-0.001	0.98	
11	*DLG2*	TC11002159.hg.1		All, n=250	0.04	**0.008**	0.35
			rs963146	AA n=104	0.02	0.34	
				AG n=64	0.05	0.08	
				GG n=5	------	------	
18	*MOCOS*	TC18000149.hg.1		All, n=250	0.03	**0.046**	0.59
			rs1057251	TT n=147	0.04	0.09	
				CC n=22	0.08	0.13	
				CT n=4	------	------	
20	*B4GALT5*	TC20000928.hg.1		All, n=250	0.01	0.73	**0.001**
			rs686237	CC n=88	-0.11	**0.03**	
				AC n=66	0.17	**0.02**	
				AA n=19	0.20	0.14	
20	*SLC9A8*	TC20000391.hg.1		All, n=250	-0.01	0.53	0.18
			rs686237	CC n=88	-0.06	0.09	
				AC n=66	0.08	0.08	
				AA n=19	-0.03	0.66	

Analyses were adjusted for age, sex and cell count. Coef: log fold change in gene expression per 10 $\mu g/m^3$ increase in NO_2 exposure; p-value: p-value for association between NO_2 exposure and gene expression; Interaction p-value: p-value for association between SNPxNO_2 and gene expression using additive effect of SNP. *n=250 for the NO_2 to gene expression association analyses and n=173 for the SNP x NO_2 to gene expression analyses.

GWIS traffic air pollution and asthma

Table 3. Significant associations of short-term diesel exhaust exposure (DE) and CpG site methylation difference (post-pre exposure) in asthmatic adults (n=16).

Chr	GWIS locus	Probe	Probe position (build 37)	CpG site location	delta FA* (post-re)	delta DE† (post-pre)	DE p-value	DE adjusted p-value‡
5	*ADCY2*	cg04119977	7,826,972	*ADCY2* (Body)	0.001	-0.019	0.0011	**0.041**
5	*ADCY2*	cg10995381	7,877,198	*MTRR* (Body)	-0.017	-0.032	0.0011	**0.041**
11	*DLG2*	cg26449294	83,169,193	*DLG2* (3'UTR)	-0.010	-0.021	$4.64x10^{-5}$	**0.017**
11	*DLG2*	cg09080874	83,284,905	*DLG2* (Body)	-0.015	-0.027	$3.01x10^{-4}$	**0.029**
11	*DLG2*	cg27373604	83,372,714	*DLG2* (5'UTR;Body)	-0.013	-0.026	0.0021	**0.041**
11	*DLG2*	cg08432013	83,393,570	*DLG2* (Body; TSS200)	-0.010	-0.025	$5.85x10^{-4}$	**0.031**
11	*DLG2*	cg02675969	83,526,604	*DLG2* (Body)	-0.010	-0.017	0.0018	**0.041**
11	*DLG2*	cg05405389	84,386,472	*DLG2* (Body)	-0.002	-0.035	0.0016	**0.041**
11	*DLG2*	cg18023263	84,403,466	*DLG2* (Body)	-0.020	-0.022	0.0013	**0.041**
11	*DLG2*	cg14716968	84,635,906	*DLG2* (TSS1500;Body)	-0.010	-0.037	$4.28x10^{-4}$	**0.029**
11	*DLG2*	cg20275558	85,338,473	*TMEM126B*; *DLG2* (TSS1500;TSS200)	0.011	0.042	$1.07x10^{-4}$	**0.020**
11	*DLG2*	cg06698742	85,359,218	*TMEM126A* (5'UTR)	0.008	0.0041	0.0014	**0.041**
18	*MOCOS*	cg19453250	33,710,783	*SLC39A6*; *ELP2* (TSS1500;Body)	-0.021	0.024	0.0019	**0.041**

*delta FA: relative methylation change post vs. pre exposure of filtered air. †delta DE: relative methylation change post vs. pre exposure of diesel exhaust.

‡Adjusted p-values using FDR method for multiple testing at a 5% level

AJRCCM Articles in Press. Published on 30-November-2016 as 10.1164/rccm.201605-1026OC

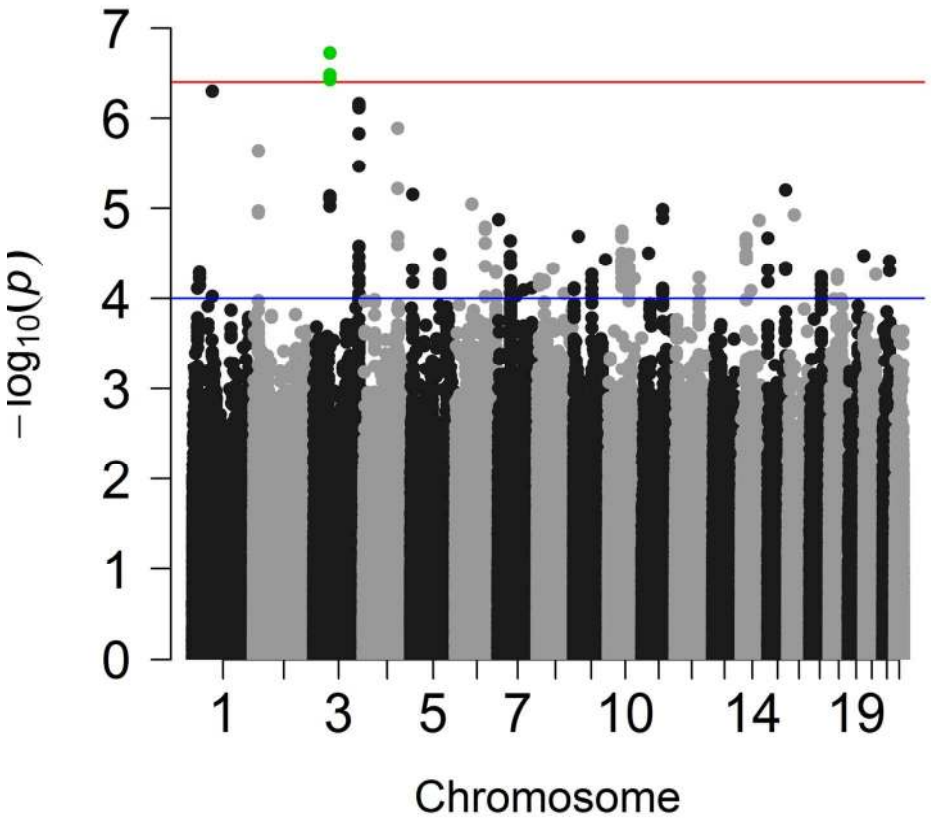


Figure 1. Manhattan plot for the discovery genome-wide interaction meta-analysis of the association between SNPxNO_2 and asthma. The horizontal red line indicates the genome-wide significance threshold when using the two-step interaction approach ($p<4x10^{-7}$). The horizontal blue line indicates the threshold for SNPs selected for look-up ($p<1x10^{-4}$, n=186). The locus, near *MAGI1* on chromosome 3p14.1, which reached genome-wide significance when using the two-step interaction approach is marked in green (rs7651862, rs11706125, rs11718057, rs13066946).
Figure 1
127x127mm (300 x 300 DPI)

AJRCCM Articles in Press. Published on 30-November-2016 as 10.1164/rccm.201605-1026OC

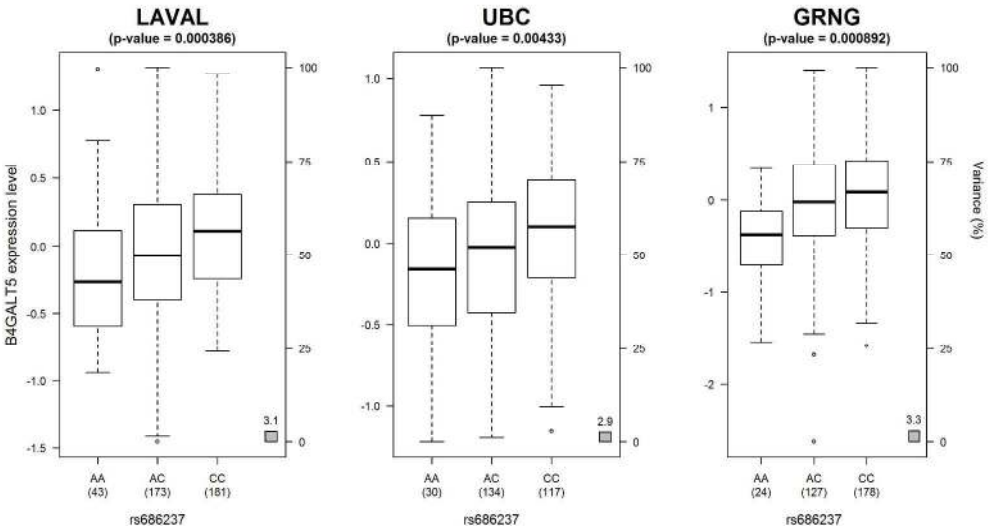


Figure 2. Gene expression levels of the B4GALT5 gene in lung tissues according to genotyping groups for SNP rs686237 (using an additive model). The left, middle, and right panels are results from Laval (n=397, p=3.86x10-4), UBC (n=281, p=0.0043) and Groningen (n=329, p=8.92x10-4), respectively, with a meta-analysis p-value of 1.18x10-17. Expression is presented for probeset 100313047_TGI_at. The y-axis represents gene expression levels in the lung. The x-axis represents the three genotyping groups for SNP rs686237 (build 37 position 48,370,734) with the number of subjects in parenthesis. The right y-axis presents the percent variance in gene expression levels explained by the genotype.

Figure 2
248x134mm (300 x 300 DPI)

Online Repository - GWIS traffic air pollution and asthma

Genome-wide interaction analysis of air pollution exposure and childhood asthma with functional follow-up

Anna Gref, Simon Kebede Merid, Olena Gruzieva, Stéphane Ballereau, Allan Becker, Tom Bellander, Anna Bergström, Yohan Bossé, Matteo Bottai, Moira Chan-Yeung, Elaine Fuertes, Despo Ierodakoniou, Ruiwei Jiang, Stéphane Joly, Meaghan Jones, Michael S. Kobor, Michal Korek, Anita L. Kozyrskyj, Ashish Kumar, Nathanaël Lemonnier, Elaina MacIntyre, Camille Ménard, David Nickle, Ma'en Obeidat, Johann Pellet, Marie Standl, Annika Sääf, Cilla Söderhäll, Carla MT. Tiesler, Maarten van den Berge, Judith M. Vonk, Hita Vora, Cheng-Jian Xu, Josep M. Antó, Charles Auffray, Michael Brauer, Jean Bousquet, Bert Brunekreef, W. James Gauderman, Joachim Heinrich, Juha Kere, Gerard H. Koppelman, Dirkje Postma, Christopher Carlsten, Göran Pershagen, Erik Melén.

ONLINE DATA SUPPLEMENT

STUDY SAMPLE DESCRIPTION

BAMSE: The Children, Allergy, Milieu, Stockholm, Epidemiological Survey (BAMSE) is a population based prospective birth cohort study with follow-up through the age of 16.(E1) Between February 1994 and November 1996 newborns were recruited at their first child health visit in predefined areas of Stockholm, Sweden (n=4,089). Infants were excluded if their family was planning to move during the first year of life, an older sibling was already enrolled, serious

AJRCCM Articles in Press. Published on 30-November-2016 as 10.1164/rccm.201605-1026OC

illness during the neonatal period or parents had insufficient knowledge of Swedish. Parental questionnaires were used to assess physician diagnosed asthma, allergic rhinitis and eczema; and episodes of wheezing at ages 1, 2, 4, 8, 12 and 16 years. At 8 and 16 years of age, all children of the BAMSE study were invited to a clinical examination, and blood samples were obtained from 2,480 children and 2,547 children respectively. At 8 years DNA was extracted from 2,033 samples after exclusion of samples with too little blood, lack of questionnaire data, or if parental consent to genetic analysis of the sample was not obtained. From these samples, all children with a doctor's diagnosis of asthma (at any time during follow-up until 8 years of age) were selected as cases (n=273) and children with no history of asthma or other allergic diseases were selected as controls (n=273). After Quality Control (QC) a total of 238 cases (asthma ever) and 246 controls were retained in the GWAS analyses.(E2) Gene expression data (16 y) was available from a subset of 263 individuals included in the MeDALL study.(E3) The study was approved by the Ethics Committee of Karolinska Institutet, Stockholm, Sweden. **Conflicts of interest:** None. **Acknowledgements:** We would like thank all the families for their participation in the BAMSE study. In addition, we would like to thank Eva Hallner, André Lauber and Sara Nilsson at the BAMSE office for invaluable support.

GINI and LISA: 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 study. A total of 3097 healthy, full-term neonates were recruited between 1997 and 1999 in Munich, Leipzig, Wesel and Bad Honnef. The participants were not pre-selected based on family history of allergic diseases.(E4) A total of 5991 mothers and their newborns were recruited into the German Infant study on the influence of

Nutrition Intervention PLUS environmental and genetic influences on allergy development (GINIplus) between September 1995 and June 1998 in Munich and Wesel. Infants with at least one allergic parent and/or sibling were allocated to the interventional study arm investigating the effect of different hydrolysed formulas for allergy prevention in the first year of life.(E5) All children without a family history of allergic diseases and children whose parents did not give consent for the intervention were allocated to the non-interventional arm. Detailed descriptions of the LISAplus and GINIplus studies have been published elsewhere.(E4, 5) Information on ever having physician-diagnosed asthma and wheeze was collected using self-administered questionnaires completed by the parents. The questionnaires were completed at 6, 12, 18 and 24 months and 4, 6, 10 years of age in the LISAplus study and 1, 2, 3, 4, 6 and 10 years in the GINIplus study asking for each year of age since the previous follow-up and for wheeze in the past 12 months at age 10 years. DNA was collected at the age 6 and 10 years. For both studies, approval by the local Ethics Committees and written consent from participant's families were obtained. **Conflicts of interest:** None. **Acknowledgements:** The authors thank all the families for their participation in the GINIplus and LISAplus studies. Furthermore, we thank all members of the GINIplus and LISAplus Study Groups for their excellent work. The LISAplus Study Group consists of the following: Helmholtz Zentrum Muenchen - German Research Center for Environment and Health, Institute of Epidemiology I, Neuherberg (Heinrich J, Wichmann HE, Sausenthaler S, Chen C-M); University of Leipzig, Department of Pediatrics (Borte M), Department of Environmental Medicine and Hygiene (Herbarth O); Department of Pediatrics, Marien-Hospital, Wesel (von Berg A); Bad Honnef (Schaaf B); UFZ-Centre for Environmental Research Leipzig-Halle, Department of Environmental Immunology (Lehmann I); IUF – Leibniz Research Institute for Environmental Medicine, Düsseldorf (Krämer U); Department of

AJRCCM Articles in Press. Published on 30-November-2016 as 10.1164/rccm.201605-1026OC

Pediatrics, Technical University, Munich (Bauer CP, Hoffman U). The GINIplus Study Group consists of the following: Helmholtz Zentrum Muenchen - German Research Center for Environmental Health, Institute of Epidemiology I, Munich (Heinrich J, Wichmann HE, Sausenthaler S, Chen C-M, Thiering E, Tiesler C, Standl M, Schnappinger M, Rzehak P); Department of Pediatrics, Marien-Hospital, Wesel (Berdel D, von Berg A, Beckmann C, Groß I); Department of Pediatrics, Ludwig Maximilians University, Munich (Koletzko S, Reinhardt D, Krauss-Etschmann S); Department of Pediatrics, Technical University, Munich (Bauer CP, Brockow I, Grübl A, Hoffmann U); IUF – Leibniz Research Institute for Environmental Medicine, Düsseldorf (Krämer U, Link E, Cramer C); Centre for Allergy and Environment, Technical University, Munich (Behrendt H).

PIAMA: the Prevention and Incidence of Asthma and Mite Allergy study (PIAMA) is a birth cohort study consisting of two parts: a placebo controlled intervention study in which the effect of mite impermeable mattress covers on the development of asthma and allergy was studied and a natural history study in which no intervention took place. Details of the study design have been published previously.(E6) Recruitment took place in 1996-1997 through prenatal clinics. A screening questionnaire was distributed to pregnant women visiting one of 52 prenatal clinics at three regions in the Netherlands. A total of 10,232 pregnant women completed a validated screening questionnaire. Mothers reporting a history of asthma, current hay fever or allergy to pets or house dust mite were defined as allergic. Based on this screening, 7,862 women were invited to participate, of whom 4,146 women (1,327 allergic and 2,819 non-allergic) gave written informed consent. Follow-up of the children took place at 3 months of age and yearly from 1 to 8 years of age. The Medical Ethical Committees of the participating institutes approved the study,

and all participants gave written informed consent. Cases were defined as a parental report of doctor's diagnosed asthma at any time between age 1 – 8 y, controls were defined a report of a negative response to this question. **Conflicts of interest:** None. **Acknowledgement:** The PIAMA birth cohort study is a collaboration of the Institute for Risk Assessment Sciences, University Utrecht (B. Brunekreef), Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht (H.A. Smit), Centre for Prevention and Health Services Research, National Institute for Public Health and the Environment, Bilthoven (A.H. Wijga), Department of Pediatrics, Division of Respiratory Medicine, Erasmus MC -Sophia, Rotterdam (J.C. de Jongste), Pulmonology (D.S. Postma) and Pediatric Pulmonology and Pediatric Allergology (G.H. Koppelman) of the University Medical Center Groningen and the Department of Immunopathology, Sanquin Research, Amsterdam (R.C. Aalberse), The Netherlands. The study team gratefully acknowledges the participants in the PIAMA birth cohort study, and all coworkers who helped conducting the medical examinations, field work and data management.

CHS: The Children's Health Study (CHS) is a longitudinal study of childhood asthma and other respiratory outcomes. It comprises of several cohorts with recruitment periods spanning from 1994 to 2003 and age at baseline ranging from age 5 to 14 years.(E7) The children were recruited from 16 southern California communities. A genome-wide association study (GWAS) was conducted based on a case-control sample of Hispanic White (HW) and non-Hispanic White (NHW) children drawn from the CHS. Stratified random sampling was used to match the controls with cases and was based on cohort, ethnicity, sex and follow up time that was frequency matched. Cases had doctor-diagnosis of asthma at study entry (as reported by their parent of guardian) or during follow-up to 2007. Study samples obtained from the buccal cells

AJRCCM Articles in Press. Published on 30-November-2016 as 10.1164/rccm.201605-1026OC

were genotyped for over 550,000 single nucleotide polymorphisms (SNPs) spanning the genome using the Illumina HumanHap550. After quality control of genotype data, such as only retaining SNPs with call rate ≥95%, 3,000 subjects were available for analysis of which 1602 were non-Hispanic whites (959 controls, 643 cases) with a baseline age ranging from 5 to 14 years. Ancestry covariates were obtained from the software STRUCTURE. These latter covariates were based on 557 ancestrally informative markers and quantified proportions of Caucasian, Native American, Asian and African American ancestry for each individual. The regional air pollution measurements were obtained from the central monitoring sites, which measured the air pollutants continuously in each of the 16 study communities. Annual average values were computed for analysis purposes. Logistic regression analysis was used to examine the interaction of NO_2 with each SNP. The model included asthma as the outcome variable and the independent variables included sex, age at baseline, community of residence, baseline environmental tobacco smoke obtained from questionnaires filled by the parents, ancestry factors, SNP, NO_2, and the interaction of the SNP with NO_2. **Conflicts of interest:** None.

CAPPS and SAGE: The Canadian Asthma Primary Prevention Study (CAPPS) is a prospective, randomized controlled study with follow-up to the age of 7 years. 545 high-risk infants (those having one first-degree relative with asthma or two first-degree relatives with other IgE mediated diseases) were randomized prior to birth in the study centers of Vancouver and Winnipeg, Canada. The multifaceted intervention included education and counseling on the risk factors of asthma, specifically dust mite and environmental tobacco smoke avoidance, and breastfeeding support. Parents completed questionnaires on respiratory symptoms and physician diagnoses at 1, 2 and 7 years. At 7 years children were examined by a pediatric allergist blinded to

intervention status and questionnaire responses; and peripheral blood was obtained from children and their parents. In this study, asthma at age 7 years was defined form the pediatric allergist's clinical exam.(E8),(E9) The Study of Asthma, Genetics and Environment (SAGE) is a population-based birth cohort. Children were identified for inclusion from a provincial healthcare registry. The study included all 13,980 children born in the province of Manitoba in 1995 with continued residence in the province through 2002. Surveys were sent to each family when children were 7 years old and, from the 3,598 responders, 723 children were selected for a nested case-control study of asthma (246 asthmatics; 477 controls). Only these children were included in the TAG collaboration, and are this included in the current study. At mean age of 9 years, children were examined by a pediatric allergist for allergic diseases, including asthma, and symptoms.(E10) **Conflicts of interest:** None. A**cknowledgements:** We acknowledge Denise Daley and the AllerGen Genetics team for assistance with CAPPS and SAGE data management and transfer.

METHODS

Air pollution assessment

Measurements of NO_2 levels were obtained from ad hoc monitoring sites selected in each study area, chosen to present the spatial distribution of air pollution levels relevant to the cohort addresses, thus including regional background, urban background and traffic sites. Measurements were performed at each site 3 times during 2 weeks in the cold, warm, and intermediate seasons, and the results were used to estimate the annual average, adjusting for

AJRCCM Articles in Press. Published on 30-November-2016 as 10.1164/rccm.201605-1026OC

temporal variation by using a centrally located background reference site. Linear models for exposure assessment at any given site within a study area were developed using geographic information system (GIS) data of land use and road traffic characteristics. The air pollution exposure levels at birth were estimated by assigning the LUR estimated concentrations to the children's home addresses at the time of birth. LUR modeling was also used to estimate individual levels of traffic related air pollution in BAMSE at 16 years of age based on addresses at the 16-years follow-up for the analyses of associations between NO_2 exposure and gene expression levels.

Asthma definitions

In the European birth cohorts (BAMSE, GINI, LISA and PIAMA), asthma was defined as physician diagnosed asthma reported at any time during follow up until 8 years of age (approximately) and was obtained by parental questionnaires. Non-asthmatics were those never having reported a doctor's diagnosis of asthma. In CHS, asthma was defined as physician diagnosed asthma at baseline (1994-2003, age range 5-14 years) or at any time during the follow up (until 2007) and was obtained by parental questionnaire or by self-report of the child at the time of their annual follow up pulmonary function test. In CAPPS, asthma was defined as a diagnosis at 7 years of age at a clinical examination by a pediatric allergist who was blinded to intervention status and questionnaire responses. In SAGE, asthma was defined as a parental report of doctor diagnosis which was confirmed by a pediatric allergist at 8 years of age. For further details see Table E2.

Online Repository - GWIS traffic air pollution and asthma

Genotyping and quality control

DNA was extracted from peripheral blood leucocytes. BAMSE, PIAMA, CAPPS and SAGE samples were genotyped using the Illumina Human610-Quad BeadChip (Illumina Inc, San Diego, CA, USA, http://www.illumina.com) and the genotyping has been described elsewhere.(E2) GINIplus and LISAplus samples were genotyped using Affymetrix Genome-wide Human SNP array 5.0 (Affymetrix, Santa Clara, CA, USA, http://www.affymetrix.com).(E16)

For CHS, DNA was extracted from buccal cells. Samples were genotyped using the Illumina HumanHap550, HumanHap550-Duo or Human610-Quad BeadChip microarrays.(E17)

SNPxNO2 interaction and asthma; Discovery phase and meta-analysis

SNPs with minor allele frequency (MAF) < 0.05 and imputation quality score (R square for MACH users or INFO for IMPUTE users) < 0.3 were excluded before the meta-analysis step. P-values for each SNPxNO_2 across studies (taking sample size and direction of effect into account) were combined for the three studies, using METAL, version 2011-03-25.(E18) R version 3.0.2(E19) was used to generate the Manhattan plots and Quantile-Quantile plots (QQ-plot). The QQ-plot was used to assess the distribution of SNP p-values and their deviation of observed associations versus expected under the null hypothesis of no association.(E20) Linkage disequilibrium (LD) between SNPs was calculated using SNAP(E21) and is based on HapMap Release 22 data using the CEU Population panel.

Adjustments for pre-defined potential confounders were made at the individual cohort level (before meta-analysis). Adjustment factors, defined using the literature, were age at last follow-

AJRCCM Articles in Press. Published on 30-November-2016 as 10.1164/rccm.201605-1026OC

up, sex, city or region where the child lived at birth (not adjusted for in GINI and LISA because only children from Munich were included), any environmental tobacco smoke exposure during the first year of life (parental report at year 1 questionnaire), and principal components for within European diversity assessed through genotype data (not adjusted for in GINI/LISA). All children included in the final analyses had a full set of confounders available. Table E1 describes data for cases and controls with a full set of confounders.

With an estimated interaction odds ratio of 1.2, equivalent to a similar range of interaction odds ratio of Glutathione S-transferase pi 1 gene (*GSTP1*) and traffic air pollution exposure in relation to childhood asthma,(E22) the sample size needed to gain 80% power is around 1,500.(E23) Logistic regression analyses for estimation of SNP main effect and NO_2 main effect on asthma was performed in using the same set of adjustment factors. Same procedure of meta-analysis as for the GWIS was applied to the SNP main effect analyses. NO_2 main effect was meta-analyzed for BAMSE, GINI/LISA and PIAMA using random effect model in STATA v13.1.

The two-step interaction approach

In step one, we tested separately in BAMSE, GINI/LISA and PIAMA the hypothesis of H_0: $\beta_{SNP} = 0$ using NO_2 as outcome in a combined set of cases and controls,(E24) which is an expansion of the traditional GxE interaction test using a case-only design. Meta-analysis of step one p-values was performed in METAL. A subset of SNPs that exceeded a meta-analysis $p < 0.05$ for the test in step one was further analyzed separately in BAMSE, GINI/LISA and PIAMA and then meta-analyzed in step two. It has been demonstrated that this two-step approach, an easily

implemented method, is more powerful than a standard interaction test for most parameter settings.(E24)

The two degree of freedom (2df) test

Using the 2 df test we jointly tested the SNP main and SNPxNO_2 interaction effects in BAMSE, GINI/LISA and PIAMA separately.(E25) A Wald test statistics, that follows a chi-squared distribution with 2 df under the H_0: $\beta_{SNP} = \beta_{SNP*NO2} = 0$, was constructed based on β_{SNP} and $\beta_{SNP*NO2}$ estimates and their corresponding 2x2 covariance matrix. The p-values from the separate cohorts were meta-analyzed in R version 3.0.2 using Fisher's method.

SNPxNO2 interaction and asthma; look-up

Adjustment factors were similar to those in the discovery phase, with the exception of environmental tobacco smoke exposure which was at 8 years of age in SAGE, no ancestry variables were adjusted for in CAPPS and SAGE, and region (community) which was not adjusted for in CHS. SNPs with MAF < 0.05 and imputation quality score ≤ 0.99 for CAPPS and SAGE, were excluded.

Direction of interaction effect and asthma risk was investigated by logistic regression analysis of NO_2 exposure on asthma, with stratification by genotype using dominant coding, adjusting for the same factors as in the SNPxNO_2 analysis.

AJRCCM Articles in Press. Published on 30-November-2016 as 10.1164/rccm.201605-1026OC

eQTL analysis in lung tissue

Genotyping was carried out using the Illumina Human1M-Duo BeadChip and whole-genome expression profiling with an Affymetrix custom array (see GEO platform GPL10379, http://www.ncbi.nlm.nih.gov/geo/). All genes located within 500 kb of the top SNPs were selected. Imputed SNP data were used. Institutional Review Board ethical approval was obtained at the three sites, and all subjects provided written informed consent.

Gene expression analyses in peripheral blood cells

RNA from 16-year-old BAMSE subjects was extracted from PAXgene tubes (PAXgeen Blood RNA Kit, Qiagen) with high quality standards, processed into biotin-labelled single-strand complementary DNA (sscDNA) according to manufacturer instructions of the WT Plus Reagent Kit and hybridized on Affymetrix Human Transcriptome Array 2.0 Genechips (HTA 2.0) for fluorometric intensity detection.(E26) Experimental design including randomization of samples into batches at crucial steps offered an efficient tracking and management of batch-related variation and afferent experimental variables. Assessment of purified molecules yield and quality (RIN(E27, 28)) was performed with state-of-the-art spectrophotometry (Trinean DropSense 96) and lab-on-a-chip microfluidic technologies (Agilent Tapestation), respectively, to ensure a robust normalization of inputs. Quality control of the gene expression data confirmed high quality with metrics beyond required thresholds. The dataset includes 45 samples from a pilot phase and 224 samples from an extended phase. Quality assessment was satisfying for 263 samples in total.(E26)

Data analysis was based on the space signal transformation robust multiarray average (SST-RMA) algorithm(E29) which combines Guanine Cytosine Count Normalization (GCCN) and Signal Space Transformation (SST) approaches, first normalizing intensities based on the probe affinity difference associated with GC content, then 'stretching' the intensity distribution by decompressing the Fold Change ratios with a power law mapping, prior to applying RMA algorithm (E30) with quantile normalization(E31). The Combat method(E32) was used to adjust for batch effect between pilot and extended phases. Linear regression was used to assess associations between NO_2 exposure and expression levels adjusting for age, sex and peripheral blood cell count.(E26)

DNA methylation analysis in the BAMSE cohort

In the BAMSE cohort epigenome-wide DNA methylation was measured in 472 Caucasian children, using DNA extracted from blood samples collected at the age of 8 years.(E26) An aliquot (500 ng) of DNA per sample underwent bisulfite conversion using the EZ-96 DNA Methylation kit (Zymo Research Corporation, Irvine, USA). Samples were plated onto 96-well plates in randomized order. Samples were processed with the Illumina Infinium HumanMethylation450 BeadChip (Illumina Inc., San Diego, USA).

Quality control of analysed samples was performed using standardized criteria. Samples were excluded in case of sample call rate <99%, colour balance >3, low staining efficiency, poor extension efficiency, poor hybridization performance, low stripping efficiency after extension and poor bisulfite conversion. We also applied multidimensional scaling (MDS) plot to evaluate gender outliers based on chromosome X data, that produced two separated clusters for male and

AJRCCM Articles in Press. Published on 30-November-2016 as 10.1164/rccm.201605-1026OC

female. We omitted 5 samples that do not belong to the distinct cluster. Furthermore, we applied median intensity plot for methylated and unmethylated intensity by using the minfi R package (3 samples below the 10.5 cutoff were excluded). All above led to exclusion of 8 samples.

Probes with a single nucleotide polymorphism in the single base extension site with a frequency of >5% were excluded, (E33) as were probes with non-optimal binding (non-mapping or mapping multiple times to either the normal or the bisulphite-converted genome), and the probe belonging to chromosome X and chromosome Y, resulting in the exclusion of 46,799 probes, leaving a total of 438,713 probes in the analysis. Furthermore, we implemented "DASEN" recommended from wateRmelon package to do signal correction and normalization.(E34)

Adjustment for cell type (estimated counts of CD8+ T cells, CD4+ T cells, NK cells, B cells, Monocytes and Granulocytes)(E35) was done using the minfi R package(E36) in the robust linear regression analysis between CpG site methylation and long-term NO_2 exposure at birth.

methQTL analyses in peripheral blood

The CpG sites investigated for association with long-term NO_2 exposure in BAMSE were also investigated for cis-methylation quantitative trait locus (cis-methQTL) effects (n=460) evaluating the eight SNPs significant in the look-up analyses. Adjustment was done for age, sex, environmental tobacco smoke exposure during first year of life, NO_2 exposure at birth, municipality, ever doctor's diagnosis of asthma up to 8 years of age, cell type (estimated counts of CD8+ T cells, CD4+ T cells, NK cells, B cells, Monocytes and Granulocytes)(E35) using the minfi R package (E36) and batch (bisulfite treatment date). A SNP was considered a methQTL if it survived 5% FDR correction for multiple testing.

DNA methylation difference by short term diesel exposure

Exposure to diesel exhaust particles (DEP, nominally, 300 μg/m3 $PM_{2.5}$) and filtered air particles (FAP) for 2 hours on two separate occasions at least two weeks apart followed a randomized crossover design. The subjects alternated between light exercise (15 min) and rest (45 min) on a stationary bike during exposure. Peripheral blood mononuclear cells (PBMCs) were collected at baseline, 6 hours, and 30 hours post-exposure. Methylation was measured using the Illumina Infinium 450K bead chip methylation array (accession number GSE56553, http://www.ncbi.nlm.nih.gov/geo/). Linear mixed effects modeling were applied to the measurements to compare post-DEP vs. pre-DEP, and post-FAP vs. pre-FAP, assuming that changes are detectable at 6 hr and persist at 30 hr post-exposure (thus, it compared DE6hr&30hr against non-DE6hr&30hr). Hits were CpG sites demonstrating significant change for the DE comparison, but not for the FA comparison. Written consent was obtained from all subjects, and the protocol was approved by the institutional review board for human studies at the University of British Columbia.

FAMTOM5 and the Human Protein Atlas

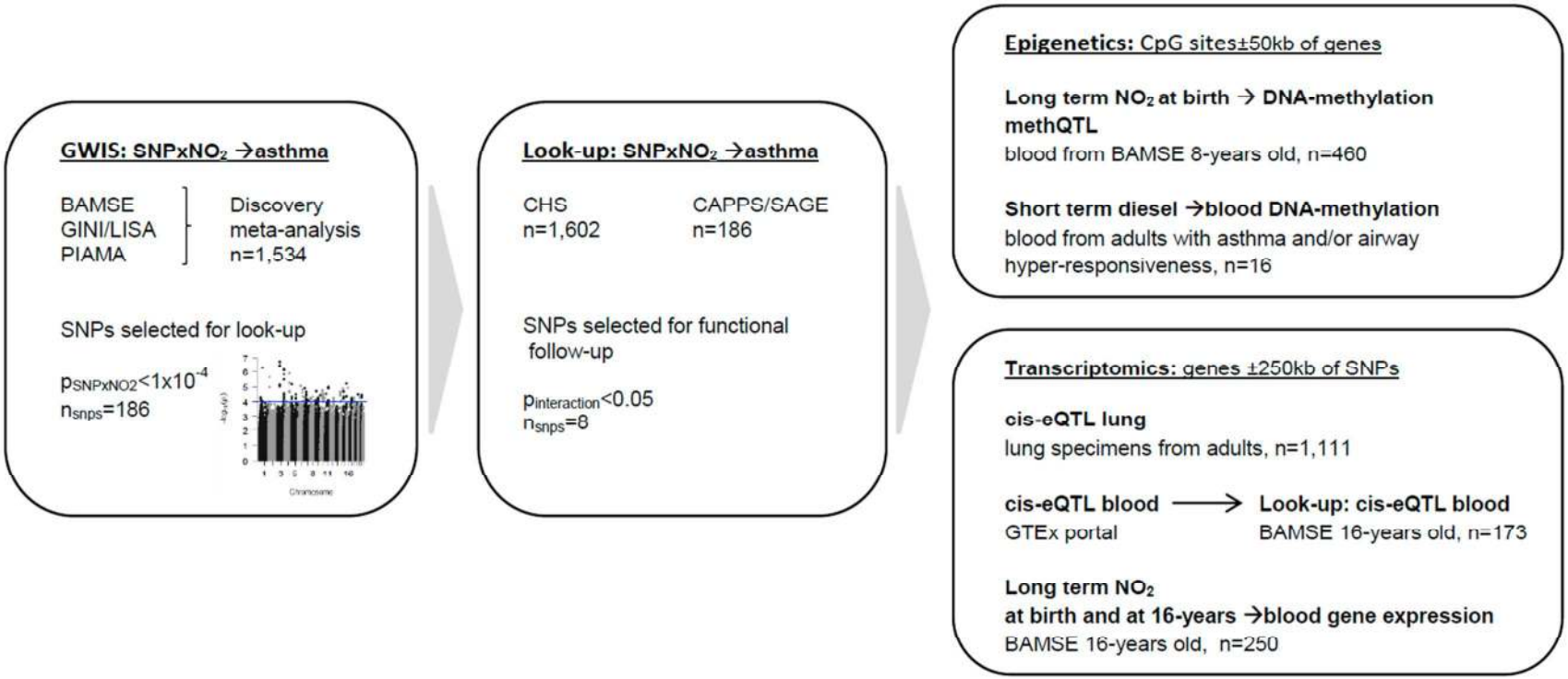
Gene expression was checked in different tissues according to the FANTOM5 data.(E37) Significant expression of *B4GALT5* is seen in most cells, including the lungs and airways, however it is specifically highly expressed in neutrophils and CD14 monocytes. *ADCY2* is highly expressed in the brain, but importantly, also found at significant levels in the fetal and adult lung and bronchi. *DLG2* expression is seen in most cells including neutrophils, regulatory T cells and

AJRCCM Articles in Press. Published on 30-November-2016 as 10.1164/rccm.201605-1026OC

lung. The Human Protein Atlas(E38) was further used to identify protein expression in normal respiratory system tissue and smooth muscle tissue. ADCY2 was expressed at medium levels in nasopharynx- and bronchial epithelial cells, lung macrophages and smooth muscle tissue (Table E14 in the online data supplement). DLG2 was expressed at low to medium levels, in nasopharynx and bronchus. B4GALT5 could not be evaluated for protein expression.

Online Repository - GWIS traffic air pollution and asthma

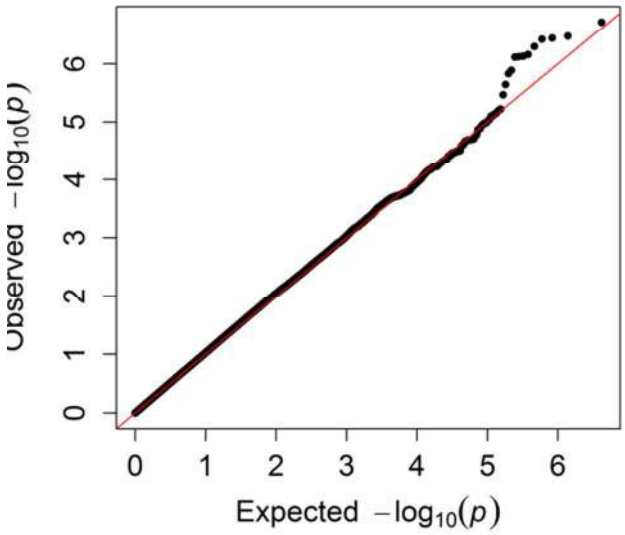
Figure E1. Overview of the study design. The procedure to identify new childhood asthma susceptibility loci that interact with traffic-related air pollution exposure. After GWIS discovery phase meta-analysis in three European cohorts, all SNPs with $p<1x10^{-4}$ for interaction were investigated for look-up evaluation in two separate North American cohorts. SNPs that were marginally significant in the look-up evaluation were further investigated in transcriptomics and epigenetics analyses.



AJRCCM Articles in Press. Published on 30-November-2016 as 10.1164/rccm.201605-1026OC

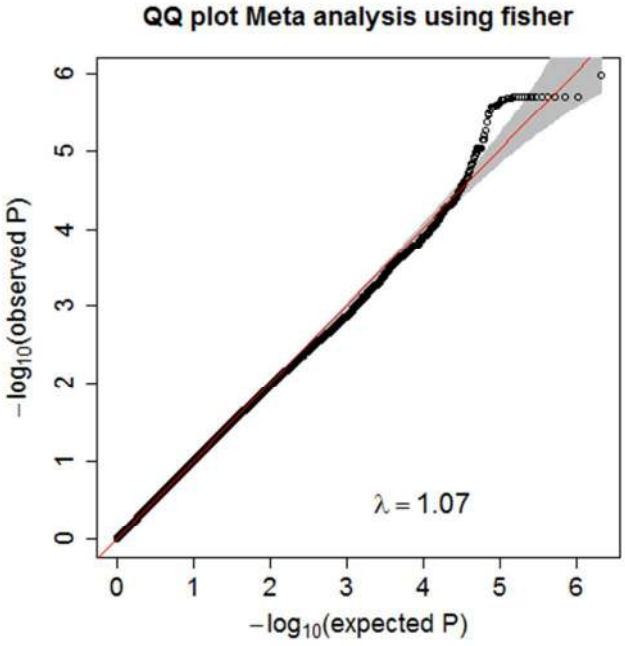
Online Repository - GWIS traffic air pollution and asthma

Figure E2. Quantile-quantile plot for the discovery genome-wide interaction meta-analysis of the association between SNPxNO_2 interaction and asthma (λ=1.03).



Online Repository - GWIS traffic air pollution and asthma

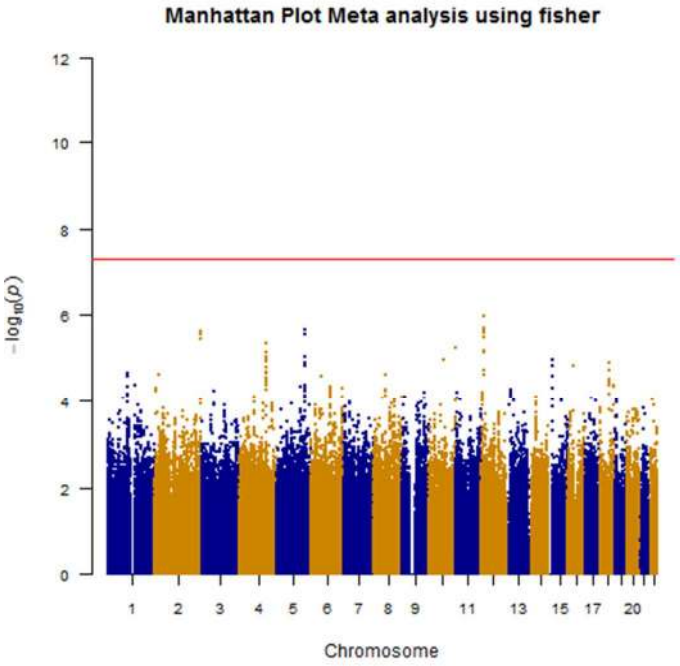
Figure E3. Quantile-quantile plot for the 2 df genome-wide meta-analysis (λ=1.07).



AJRCCM Articles in Press. Published on 30-November-2016 as 10.1164/rccm.201605-1026OC

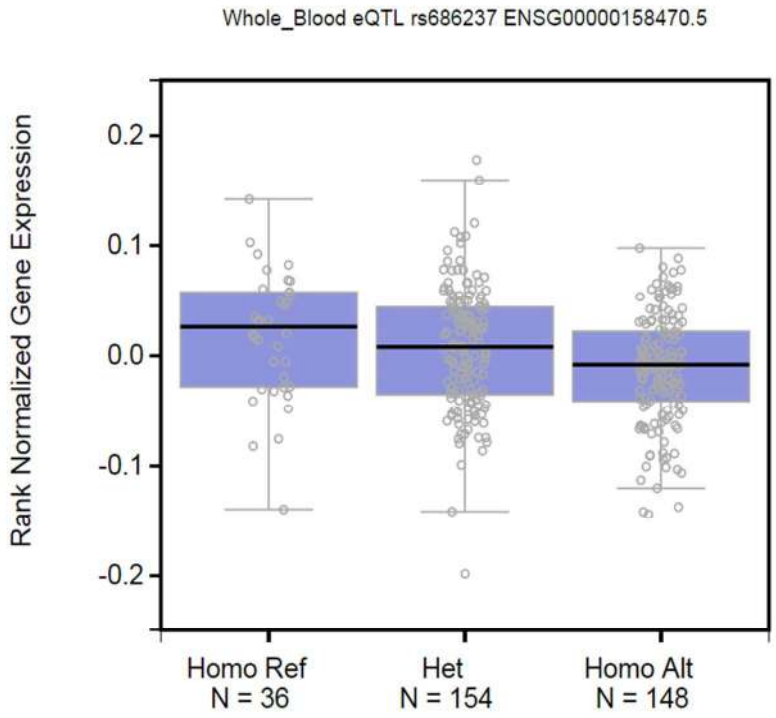
Online Repository - GWIS traffic air pollution and asthma

Figure E4. Manhattan plot for the 2 df genome-wide meta-analysis. The horizontal red line indicates the genome-wide significance threshold ($p<1x10^{-7}$).



Online Repository - GWIS traffic air pollution and asthma

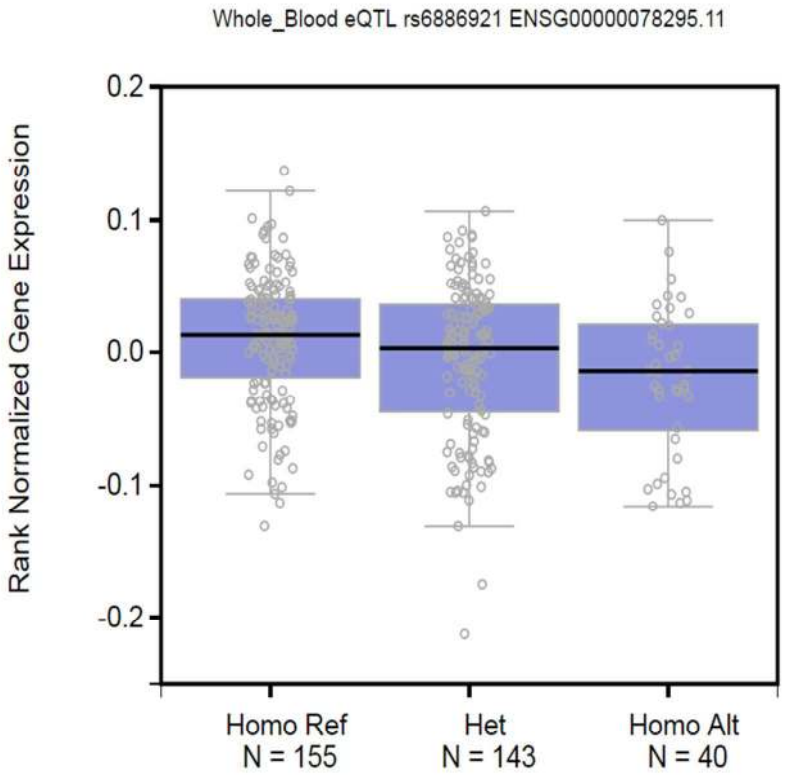
Figure E5. Gene expression levels of the *B4GALT5* gene in whole blood according to genotyping groups for SNP rs686237 (using an additive model) investigated in the GTEx Portal (n=338), $p=4.00x10^{-4}$. The x-axis represents the three genotyping groups for SNP rs686237 with the number of subjects. Homo Ref: AA, Het:AC, Homo Alt: CC.



AJRCCM Articles in Press. Published on 30-November-2016 as 10.1164/rccm.201605-1026OC

Online Repository - GWIS traffic air pollution and asthma

Figure E6. Gene expression levels of the *ADCY2* gene in whole blood according to genotyping groups for SNP rs6886921 (using an additive model) investigated in the GTEx Portal (n=338), $p=4.50x10^{-4}$. The x-axis represents the three genotyping groups for SNP rs6886921 with the number of subjects. Homo Ref: CC, Het:CT, Homo Alt: TT.



Online Repository - GWIS traffic air pollution and asthma

Table E1. Characteristics of the children in the studied cohorts.

Discovery	Asthmatics	Never asthmatics	Total	Age at last follow-up mean years (min-max)	Boys (%)	Tobacco smoke exposure* (%)	NO_2 exposure levels ($\mu g/m^3$) mean/median (min-max/5th-95th percentile)
BAMSE	235	246	481	8.3 (7.4-10.5)	265 (55)	88 (18)	13.56 / 11.97 (6.0-31.8 / 7.6-23.1)
GINI/LISA	64	661	725	10.2 (9.4-11.3)	390 (54)	127 (18)	21.6 / 20.6 (11.5-61.1 / 13.9-30. 9)
PIAMA	155	173	328	8.1 (7.8-9.6)	173 (53)	74 (23)	25.0 / 25.9 (12.6-54.6 / 14.0-37.9)
Discovery total	454	1,080	1,534				

Look-up	Asthmatics	Never asthmatics	Total	Age at last follow-up mean years (min-max)	Boys (%)	Tobacco smoke exposure (%)	NO_2 exposure levels ($\mu g/m^3$) mean/median (min-max/5th-95th percentile)
CHS	643	959	1,602	8.8 (5.2-14.3)	846 (53)	205 (13)	20.7 / 19.6 (4.2-40.8 / 4.6-38.0)
CAPPS/SAGE	49	137	186	7.4 (7.0-8.0)†	106 (57)	40 (22)‡	15.4 / 13.4 (4.5-55.2 / 5.8-35.3)
Total	1,146	2,176	3,322				

*Any environmental tobacco smoke exposure during first year of life (parental reported at year 1 questionnaire). †7 years of age in CAPPS, 8 years of age in SAGE. ‡first year of life for CAPPS, year 8 for SAGE.

AJRCCM Articles in Press. Published on 30-November-2016 as 10.1164/rccm.201605-1026OC

Online Repository - GWIS traffic air pollution and asthma

Table E2. Study characteristics genotyping and imputation procedure, traffic air pollution exposure assessment method, definitions and prevalence of outcomes, as well as statistical software used.

	Discovery cohorts			**Look-up cohorts**	
Cohort characteristics	**BAMSE**	**GINI/LISA**	**PIAMA**	**CHS**	**CAPPS/SAGE**
Study design	Population based birth cohort.	Population based birth cohort (with nutrition intervention for GINI)	Population based birth cohort (with mattress cover intervention and allergic/non-allergic parents)	Stratified random sampling was used to match the controls with cases and was based on cohort, ethnicity, sex and follow-up time (frequency matched).	Birth cohort with asthma intervention (high risk infants)/ Population based birth cohort
Age at enrolment	Newborns	Pregnant women	Pregnant women	Ranging from age 5-14yrs with a mean of 9 years	Pregnant women/ Newborns
Population source (area)	Stockholm, Sweden	Munich, Wesel/ Munich, Wesel, Bad Honnef and Leipzig, Germany	Greater Groningen, Bilthoven, Wageningen and surroundings and greater Rotterdam, Netherlands	Southern California	Vancouver and Winnipeg, Canada/ Manitoba, Canada
Enrolment period	1994-1996	1995-1998/1997-1999	1996-1997	1993, 1996, 2003	1995
Cohort recruitment	Community population	Maternity hospitals/ Obstetrical	Midwife practices	From 16 school	Parental clinics/Provincial

Online Repository - GWIS traffic air pollution and asthma

	register	clinics		communities (3 separate cohorts)	health care registry.
Total number of recruited children	4,089	5,991/3,097	4,146	~12,000, 3,000 in GWAS, 1,788 non-Hispanic white in GWAS	549/16,320
Follow-up time points (year of life)	1,2,4,8,12,16	1,2,3,4,6,10/0.5,1,1.5,2,4,6,10	1,2,3,4,5,6,7,8	Annual from age 10 to 18 in 1993 and 1996 cohorts, annual from age 5 to 17 in 2003 cohort	1,2,7/8
Assessment of outcome and covariates	Parental reported by questionnaires	Parental reported by questionnaires	Parental reported by questionnaires	Parental reported by questionnaire	Parental reported by questionnaires and confirmation of diagnoses by pediatric allergist
Phenotype definitions					
Asthma cases	Ever having a doctor's diagnosed asthma up to age 8 years. Parental questionnaire assessing physician diagnosed	Ever having a doctor's diagnosed asthma up to age 10. Parental questionnaire assessing physician diagnosed asthma.	Ever having a doctor's diagnosed asthma up to age 8. Parental questionnaire assessing physician diagnosed asthma.	Ever having a doctor's diagnosis as of 2007, based on parental or child report.	Asthma diagnosis at 7 years of age at the clinical examination by a pediatric allergist. /Asthma diagnosis at 8 years of age. Parent report of physician diagnosed asthma, confirmed

AJRCCM Articles in Press. Published on 30-November-2016 as 10.1164/rccm.201605-1026OC

	asthma.				by pediatric allergist.
Healthy controls	Never having a doctor's diagnosed asthma or allergic disease up to age 8	Never having a doctor's diagnosed asthma up to age 10	Never having a doctor's diagnosed asthma up to age 8	Never having a doctor's diagnosed of asthma as of 2007	No asthma diagnosis at 7 years of age./ No asthma diagnosis at 8 years of age
Traffic air pollution					
Estimation model	LUR	LUR	LUR	Outdoor air pollution monitoring stations in each of the study communities	LUR
Time of measurements	2009	Munich, Augsburg and small nearby towns sampled for three two-week intervals October 2008-November 2009	March 1999-July 2000	Continuous measurements from 1994 onward in each study community	Vancouver: Spring and fall 2003/ Winnipeg: 2007
Genotyping					
Criteria for selection of genotyping (GWAS)	Asthma case-control n=505	Children from Munich n=1,511	Asthma case-control n=404	Asthma case-control. Stratified random sampling was used to match the controls with cases and was based on cohort, ethnicity, sex and follow-	Asthma case-control n=956

Online Repository - GWIS traffic air pollution and asthma

				up time (frequency matched) n=1,788	
Genotyping platform	Illumina 610-Quad BeadChip	Affymetrix 5.0	Illumina 610-Quad Beadchip	Illumina HumanHap550, HumanHap550-Duo or Human610-Quad BeadChip	Illumina 610-Quad BeadChip
Genetic Quality control before imputation					
SNP call rate threshold	97%	95%	95%	95%	95%
HWE p-value threshold	1 x 10^{-4} in controls	1 x 10^{-5}	1 x 10^{-4} in controls	1 x 10^{-5} in controls	1 x 10^{-4}
MAF threshold	5%	1%	1%	No exclusions for any rof the look-up SNPs	1%
Other exclusion criteria	-	-	Ethnicity	-	SNPs with >2 Mendelian errors
Number of SNPs after filtering	515,695	357,125	516,527	172 (of 186 selected for look-up evaluation)	122 (of 186 selected for look-up evaluation)
Imputation					
Imputation software	MACH	IMPUTE 2	IMPUTE V2	MACH	MACH

AJRCCM Articles in Press. Published on 30-November-2016 as 10.1164/rccm.201605-1026OC

Online Repository - GWIS traffic air pollution and asthma

NCBI build	36	36	36	36	36
Reference panel	Hap Map release 22	Hap Map release 22	Hap Map release 22	HapMap release 22	Hap Map release 22
Number of SNPs after imputation	2,180,015	2,619,389	2,197,335	NA	NA
Inclusion criteria					
Ethnicity	Caucasian	Caucasian	Caucasian	Non-Hispanic white	Caucasian
Genotyping call rate threshold	97%	95%	95%	95%	97%
Other exclusion criteria	Twins or any multiple births	Heterozygosity thresholds: Mean ±4 SD; sex discrepancies	Heterozygosity thresholds: Mean ±4 SD; sex discrepancies	-	Heterozygosity thresholds: Mean ±3 SD; samples had to pass gender and Mendelian transmission error checks; Twins or duplicate samples
Genotype-phenotype association software and version	ProbABELv0.1-3	ProbABEL v0.1-9e	ProbABEL v0.1-9e and R	SAS v9.4	STATA v13.1

Online Repository - GWIS traffic air pollution and asthma

Table E3. List of the top SNPs ($p<1x10^{-4}$, n=186) selected for look-up from the discovery phase genome-wide interaction meta-analysis of the association between SNPxNO_2 interaction and asthma, ordered by chromosome location.

Chr	SNP	position	A1	A2	Nearest gene	Discovery GWIS meta-analysis p-value n=1,534	Heterogeneity p-value
1	rs2205722	20481895	A	C	*UBXN10,VWA5B1*	$7.73x10^{-5}$	0.8705
1	rs7515342	29652302	A	G	*PTPRU,MATN1*	$5.32x10^{-5}$	0.8765
1	rs7556278	29652615	C	T	*PTPRU,MATN1*	$5.17x10^{-5}$	0.8795
1	rs7547385	29652625	C	G	*PTPRU,MATN1*	$7.22x10^{-5}$	0.8736
1	rs7521309	29653160	C	T	*PTPRU,MATN1*	$6.03x10^{-5}$	0.8932
1	rs4654349	29653808	C	T	*PTPRU,MATN1*	$5.91x10^{-5}$	0.8206
1	rs4654350	29653811	C	G	*PTPRU,MATN1*	$5.27x10^{-5}$	0.8282
1	rs10518644	80652485	A	G	*LOC646526,LOC100129325*	$5.05x10^{-7}$	0.9592
1	rs12025147	80689103	A	C	*LOC646526,LOC100129325*	$9.50x10^{-5}$	0.8376
2	rs11096550	18722984	C	G	*NT5C1B,FLJ41481*	$1.07x10^{-5}$	0.2737
2	rs16985416	18729707	A	G	*NT5C1B,FLJ41481*	$1.12x10^{-5}$	0.2901
2	rs4614937	18731669	A	G	*NT5C1B,FLJ41481*	$2.27x10^{-6}$	0.0638
3	rs11718057	65069823	A	T	*LOC730057,MAGI1*	**$3.59x10^{-7}$**	0.9077
3	rs11706125	65069843	A	G	*LOC730057,MAGI1*	**$3.33x10^{-7}$**	0.9017
3	rs7651862	65069950	G	T	*LOC730057,MAGI1*	**$1.87x10^{-7}$**	0.8372
3	rs13066946	65070988	A	G	*LOC730057,MAGI1*	**$3.77x10^{-7}$**	0.9088
3	rs2371862	65074490	A	C	*LOC730057,MAGI1*	$7.26x10^{-6}$	0.7745
3	rs2170573	65074846	A	G	*LOC730057,MAGI1*	$7.92x10^{-6}$	0.7724
3	rs2128406	65075037	C	T	*LOC730057,MAGI1*	$7.81x10^{-6}$	0.7687
3	rs9873349	65079771	A	C	*LOC730057,MAGI1*	$9.49x10^{-6}$	0.7332
3	rs939441	181518859	C	T	*LOC647249,TTC14*	$9.67x10^{-5}$	0.7306
3	rs2878951	181526858	C	T	*LOC131054,TTC14*	$2.60x10^{-5}$	0.7011
3	rs1464358	181527268	C	T	*LOC131054,TTC14*	$2.67x10^{-5}$	0.6883
3	rs10937030	181527552	A	T	*LOC131054,TTC14*	$9.81x10^{-5}$	0.7349

AJRCCM Articles in Press. Published on 30-November-2016 as 10.1164/rccm.201605-1026OC

3	rs7431270	181529402	A	G	*LOC131054,TTC14*	$3.42x10^{-5}$	0.8498
3	rs1356909	181531444	G	T	*LOC131054,TTC14*	$4.16x10^{-5}$	0.8554
3	rs13325203	181533917	C	T	*LOC131054,TTC14*	$4.45x10^{-5}$	0.8565
3	rs9810983	181546287	C	T	*LOC131054,TTC14*	$4.53x10^{-5}$	0.8535
3	rs4854972	181550117	A	G	*LOC131054,TTC14*	$4.60x10^{-5}$	0.852
3	rs10937032	181550599	A	G	*LOC131054,TTC14*	$8.88x10^{-5}$	0.8702
3	rs1402227	181554680	G	T	*LOC131054,TTC14*	$6.74x10^{-5}$	0.8188
3	rs1464359	181556243	A	G	*LOC131054,TTC14*	$6.95x10^{-5}$	0.8216
3	rs9871421	181561017	A	C	*LOC131054,TTC14*	$6.03x10^{-5}$	0.9009
3	rs7634356	181630586	C	T	*LOC131054,TTC14*	$7.68x10^{-7}$	0.8948
3	rs1533700	181646575	A	T	*LOC131054,TTC14*	$6.93x10^{-7}$	0.8877
3	rs9823620	181654283	C	T	*LOC131054,TTC14*	$1.48x10^{-6}$	0.996
3	rs982698	181702681	C	T	*LOC131054,TTC14*	$7.40x10^{-7}$	0.8862
3	rs12497999	181767441	C	T	*LOC131054,TTC14*	$7.54x10^{-7}$	0.8861
3	rs12496529	181786971	A	C	*LOC131054,TTC14*	$3.41x10^{-6}$	0.9849
4	rs3910954	138821160	A	G	*PCDH18,LOC641365*	$2.05x10^{-5}$	0.2357
4	rs3843891	138828912	A	C	*PCDH18,LOC641365*	$2.49x10^{-5}$	0.2266
4	rs7672176	138913964	A	C	*PCDH18,LOC641365*	$6.03x10^{-6}$	0.1673
4	rs7670760	138936781	C	T	*PCDH18,LOC641365*	$1.29x10^{-6}$	0.1705
5	rs727432	7716078	G	T	*ADCY2*	$6.67x10^{-5}$	0.9545
5	rs4143882	7717364	A	G	*ADCY2*	$4.75x10^{-5}$	0.9406
5	rs6886921	7718539	C	T	*ADCY2*	$7.03x10^{-6}$	0.9787
5	rs4266448	116700966	G	T	*RPS17P2,LOC728342*	$5.43x10^{-5}$	0.6054
5	rs11749394	116708435	G	T	*RPS17P2,LOC728342*	$5.47x10^{-5}$	0.6058
5	rs10061651	116708519	A	C	*RPS17P2,LOC728342*	$5.98x10^{-5}$	0.6235
5	rs4457117	116708659	C	T	*RPS17P2,LOC728342*	$6.00x10^{-5}$	0.6271
5	rs4443456	116708696	C	T	*RPS17P2,LOC728342*	$6.12x10^{-5}$	0.6251
5	rs6595040	116708999	C	T	*RPS17P2,LOC728342*	$6.17x10^{-5}$	0.6255
5	rs4623185	116709042	A	G	*RPS17P2,LOC728342*	$3.20x10^{-5}$	0.6703
5	rs4448037	116709139	C	T	*RPS17P2,LOC728342*	$6.70x10^{-5}$	0.6216

5	rs6860005	116709836	C	T	*RPS17P2,LOC728342*	$6.20x10^{-5}$	0.6257
5	rs4458619	116711165	C	T	*RPS17P2,LOC728342*	$6.07x10^{-5}$	0.6272
5	rs13159487	116711565	A	G	*RPS17P2,LOC728342*	$6.01x10^{-5}$	0.6281
5	rs10069843	116711962	A	G	*RPS17P2,LOC728342*	$6.83x10^{-5}$	0.6225
5	rs10039799	116712524	C	T	*RPS17P2,LOC728342*	$6.90x10^{-5}$	0.6229
5	rs4259204	116713920	A	G	*RPS17P2,LOC728342*	$6.04x10^{-5}$	0.63
5	rs4566827	116714196	A	G	*RPS17P2,LOC728342*	$6.21x10^{-5}$	0.6295
6	rs16895780	65560035	C	T	*LOC100130393,LOC727977*	$9.03x10^{-6}$	0.538
6	rs9489520	119187072	A	G	*C6orf204,ASF1A*	$4.39x10^{-5}$	0.9041
6	rs601575	119526519	C	G	*FAM184A,MAN1A1*	$1.71x10^{-5}$	0.5923
6	rs660682	119526898	A	G	*FAM184A,MAN1A1*	$2.42x10^{-5}$	0.5975
6	rs6916978	119539264	A	G	*MAN1A1*	$1.61x10^{-5}$	0.4681
6	rs2558	119542373	A	G	*MAN1A1*	$9.59x10^{-5}$	0.8643
6	rs12201119	162514036	A	G	*PARK2,PARK2*	$5.19x10^{-5}$	0.9753
6	rs9456749	162518420	A	G	*PARK2,PARK2*	$5.07x10^{-5}$	0.7625
6	rs12205861	162518803	C	T	*PARK2,PARK2*	$1.00x10^{-4}$	0.9644
6	rs12208027	162522787	C	G	*PARK2,PARK2*	$9.22x10^{-5}$	0.9099
6	rs9355987	162525256	C	T	*PARK2,PARK2*	$9.73x10^{-5}$	0.9271
7	rs1713918	2910713	A	C	*CARD11*	$1.33x10^{-5}$	0.4129
7	rs11765988	50385016	A	G	*LOC100132224,IKZF1*	$2.27x10^{-5}$	0.5979
7	rs7800411	50386119	C	T	*LOC100132224,IKZF1*	$3.38x10^{-5}$	0.6609
7	rs12719039	50386443	C	T	*LOC100132224,IKZF1*	$3.89x10^{-5}$	0.7561
7	rs3807552	50578236	A	G	*DDC*	$7.13x10^{-5}$	0.766
7	rs3829897	50597258	G	T	*DDC*	$7.16x10^{-5}$	0.1999
7	rs6593010	50597382	A	G	*DDC*	$4.12x10^{-5}$	0.3377
7	rs10278338	50597764	C	T	*DDC*	$4.15x10^{-5}$	0.3392
7	rs11489734	50603224	A	T	*DDC,GRB10*	$6.45x10^{-5}$	0.2824
7	rs11238214	50603512	A	G	*DDC,GRB10*	$8.57x10^{-5}$	0.2526
7	rs13228274	50603895	A	T	*DDC,GRB10*	$8.64x10^{-5}$	0.2528
7	rs1839740	50613774	C	T	*DDC,GRB10*	$8.70x10^{-5}$	0.2543

AJRCCM Articles in Press. Published on 30-November-2016 as 10.1164/rccm.201605-1026OC

7	rs12669770	50623828	A	G	*GRB10,GRB10,GRB10*	$8.94x10^{-5}$	0.2556
7	rs4245555	50628903	C	T	*GRB10,GRB10,GRB10*	$7.37x10^{-5}$	0.8823
7	rs884843	73083725	A	G	*ELN,ELN,ELN,ELN*	$9.40x10^{-5}$	0.8952
7	rs362726	102994470	C	T	*RELN*	$8.07x10^{-5}$	0.08233
7	rs13223489	136419075	C	T	*LOC100128744*	$7.66x10^{-5}$	0.1109
8	rs2945882	8159034	A	G	*FLJ10661,PRAGMIN*	$6.13x10^{-5}$	0.1633
8	rs2955551	8160844	A	G	*FLJ10661,PRAGMIN*	$7.17x10^{-5}$	0.1774
8	rs12681261	28495356	C	T	*FZD3,EXTL3*	$6.77x10^{-5}$	0.7136
8	rs164658	28497485	A	G	*FZD3,EXTL3*	$6.32x10^{-5}$	0.7467
8	rs17265947	63901079	A	G	*NKAIN3*	$4.65x10^{-5}$	0.8752
8	rs17336727	107438950	A	T	*LOC100128259,OXR1*	$8.84x10^{-5}$	0.6875
9	rs10812133	2490687	A	G	*SMARCA2,FLJ35024*	$7.69x10^{-5}$	0.8804
9	rs10491713	2496236	G	T	*SMARCA2,FLJ35024*	$7.99x10^{-5}$	0.9203
9	rs2383131	19892822	C	T	*SLC24A2,SMNP*	$2.03x10^{-5}$	0.3345
9	rs11142863	73325288	C	T	*TRPM3,TMEM2*	$7.91x10^{-5}$	0.1689
9	rs11142864	73325468	A	C	*TRPM3,TMEM2*	$7.89x10^{-5}$	0.1687
9	rs17056968	73345792	C	T	*TRPM3,TMEM2*	$9.49x10^{-5}$	0.1526
9	rs10869033	73355863	G	T	*TRPM3,TMEM2*	$5.43x10^{-5}$	0.1853
9	rs10781028	73358763	C	T	*TRPM3,TMEM2*	$9.18x10^{-5}$	0.1545
9	rs12685824	73361662	A	T	*TRPM3,TMEM2*	$6.36x10^{-5}$	0.1973
9	rs539215	128101333	A	C	*PBX3,FAM125B*	$3.70x10^{-5}$	0.8072
10	rs1194673	53811658	A	G	*DKK1,LOC644522*	$7.45x10^{-5}$	0.4049
10	rs1149776	53812481	A	G	*DKK1,LOC644522*	$7.25x10^{-5}$	0.4061
10	rs1149772	53815310	C	T	*DKK1,LOC644522*	$6.12x10^{-5}$	0.4151
10	rs1149769	53816103	C	G	*DKK1,LOC644522*	$5.09x10^{-5}$	0.4274
10	rs1194671	53817795	C	T	*DKK1,LOC644522*	$4.57x10^{-5}$	0.4334
10	rs1194670	53818050	A	C	*DKK1,LOC644522*	$4.39x10^{-5}$	0.4357
10	rs1194668	53818632	A	G	*DKK1,LOC644522*	$3.11x10^{-5}$	0.476
10	rs6480837	53819249	A	C	*DKK1,LOC399774*	$3.87x10^{-5}$	0.4431
10	rs1194664	53820821	C	T	*DKK1,LOC399774*	$3.76x10^{-5}$	0.4435

10	rs1194662	53823021	C	T	*DKK1,LOC399774*	$1.95x10^{-5}$	0.5658
10	rs1194661	53823035	A	G	*DKK1,LOC399774*	$2.00x10^{-5}$	0.563
10	rs1194659	53823557	C	T	*LOC644522,LOC399774*	$1.98x10^{-5}$	0.5638
10	rs1660793	53827025	C	T	*LOC644522,LOC399774*	$2.03x10^{-5}$	0.565
10	rs1621210	53827070	C	T	*LOC644522,LOC399774*	$1.78x10^{-5}$	0.5786
10	rs1660792	53827231	A	C	*LOC644522,LOC399774*	$2.00x10^{-5}$	0.5664
10	rs1733704	53827622	C	T	*LOC644522,LOC399774*	$2.02x10^{-5}$	0.5661
10	rs1733706	53828582	A	G	*LOC644522,LOC399774*	$2.10x10^{-5}$	0.5737
10	rs12412762	81897450	C	T	*PLAC9,ANXA11*	$4.88x10^{-5}$	0.7776
10	rs17100316	81903317	A	G	*ANXA11,ANXA11*	$4.72x10^{-5}$	0.7723
10	rs12769764	81907093	C	G	*ANXA11,ANXA11*	$3.19x10^{-5}$	0.8254
10	rs3862518	81907937	C	T	*ANXA11,ANXA11*	$3.43x10^{-5}$	0.83
10	rs12268619	81910640	A	C	*ANXA11,ANXA11*	$6.29x10^{-5}$	0.5474
10	rs2304410	81911790	C	T	*ANXA11,ANXA11*	$6.72x10^{-5}$	0.5515
10	rs10466226	81914035	A	G	*ANXA11,ANXA11*	$6.92x10^{-5}$	0.5543
10	rs10466228	81914353	A	G	*ANXA11,ANXA11*	$6.89x10^{-5}$	0.5514
10	rs11201966	81921760	C	T	*ANXA11,ANXA11*	$6.92x10^{-5}$	0.557
10	rs11201972	81922660	C	T	*ANXA11,ANXA11*	$6.98x10^{-5}$	0.5565
10	rs12769115	81923481	A	G	*ANXA11,ANXA11*	$8.62x10^{-5}$	0.6426
10	rs12763392	81924975	C	T	*ANXA11,ANXA11*	$6.37x10^{-5}$	0.686
10	rs3851055	81928076	C	T	*ANXA11,ANXA11*	$7.09x10^{-5}$	0.6603
10	rs12256429	81928612	C	T	*ANXA11,ANXA11*	$4.56x10^{-5}$	0.7135
10	rs12779955	81930844	C	T	*ANXA11,ANXA11*	$7.30x10^{-5}$	0.6549
10	rs2244524	104476964	C	T	*SFXN2*	$6.06x10^{-5}$	0.3672
11	rs10834971	26337381	A	C	*TMEM16C*	$3.12x10^{-5}$	0.4219
11	rs1545863	83381380	G	T	*DLG2*	$7.79x10^{-5}$	0.875
11	rs1545864	83383163	C	G	*DLG2*	$1.05x10^{-5}$	0.9265
11	rs12418356	83385605	A	G	*DLG2*	$1.03x10^{-5}$	0.9283
11	rs1601091	83388136	C	T	*DLG2*	$7.73x10^{-5}$	0.8753
11	rs1384749	83397130	G	T	*DLG2*	$8.15x10^{-5}$	0.8673

AJRCCM Articles in Press. Published on 30-November-2016 as 10.1164/rccm.201605-1026OC

11	rs11233881	83409188	A	C	*DLG2*	$1.29x10^{-5}$	0.9346
11	rs963146	83423444	A	G	*DLG2*	$8.61x10^{-5}$	0.9208
11	rs1384751	83432571	A	C	*DLG2*	$8.84x10^{-5}$	0.9223
12	rs34577	95049250	A	G	*LOC100132930,ELK3*	$8.18x10^{-5}$	0.9316
12	rs34579	95050134	C	T	*LOC100132930,ELK3*	$5.91x10^{-5}$	0.6654
14	rs11157090	39421942	G	T	*FBXO33,LOC644919*	$3.47x10^{-5}$	0.9558
14	rs7158182	39423507	A	G	*FBXO33,LOC644919*	$2.48x10^{-5}$	0.9898
14	rs10150213	39423615	A	T	*FBXO33,LOC644919*	$3.48x10^{-5}$	0.9515
14	rs8004765	39424373	A	G	*FBXO33,LOC644919*	$3.35x10^{-5}$	0.9543
14	rs11844981	39429789	A	G	*FBXO33,LOC644919*	$2.18x10^{-5}$	0.9907
14	rs10151130	39441365	C	T	*FBXO33,LOC644919*	$3.24x10^{-5}$	0.9644
14	rs1957231	39442479	A	C	*FBXO33,LOC644919*	$3.20x10^{-5}$	0.9646
14	rs17180573	39443197	A	T	*FBXO33,LOC644919*	$3.17x10^{-5}$	0.9649
14	rs17180580	39443302	A	G	*FBXO33,LOC644919*	$3.36x10^{-5}$	0.9661
14	rs10149674	39443919	G	T	*FBXO33,LOC644919*	$3.19x10^{-5}$	0.9655
14	rs10137555	39444113	A	G	*FBXO33,LOC644919*	$2.13x10^{-5}$	0.9916
14	rs11847742	39447796	C	T	*FBXO33,LOC644919*	$3.21x10^{-5}$	0.9659
14	rs1957229	39448355	A	G	*FBXO33,LOC644919*	$3.22x10^{-5}$	0.9662
14	rs1957221	39464798	A	T	*FBXO33,LOC644919*	$3.72x10^{-5}$	0.9689
14	rs10144664	39465191	C	T	*FBXO33,LOC644919*	$3.73x10^{-5}$	0.9689
14	rs10150328	58450585	C	T	*LOC440181,AKR1B1P5*	$8.24x10^{-5}$	0.9966
14	rs4900108	91823837	A	C	*CPSF2,SLC24A4*	$1.35x10^{-5}$	0.9069
15	rs999842	20551713	A	G	*CYFIP1*	$2.12x10^{-5}$	0.6508
15	rs7179062	20553025	C	G	*CYFIP1*	$4.85x10^{-5}$	0.2729
15	rs7179447	20553224	C	G	*CYFIP1*	$6.53x10^{-5}$	0.2907
15	rs7182254	91715858	C	T	*UNQ9370,LOC283682*	$6.33x10^{-6}$	0.08291
15	rs1489135	91717789	A	T	*UNQ9370,LOC283682*	$4.54x10^{-5}$	0.03214
15	rs12439678	91718676	C	T	*UNQ9370,LOC283682*	$4.81x10^{-5}$	0.06388
16	rs237191	26580307	A	G	*HS3ST4,C16orf82*	$1.18x10^{-5}$	0.1072
17	rs4794298	47151791	A	G	*CA10,CA10*	$6.10x10^{-5}$	0.8789

Online Repository - GWIS traffic air pollution and asthma

17	rs7223543	47156558	A	G	*CA10,CA10*	$8.17x10^{-5}$	0.8288
17	rs1917998	47160827	A	C	*CA10,CA10*	$6.92x10^{-5}$	0.9682
17	rs11869209	47163241	A	C	*CA10,CA10*	$7.06x10^{-5}$	0.9335
17	rs7214924	47165513	G	T	*CA10,CA10*	$9.60x10^{-5}$	0.9593
17	rs8074550	47168474	A	T	*CA10,CA10*	$5.73x10^{-5}$	0.9904
18	rs12455842	32096284	C	T	*MOCOS*	$6.10x10^{-5}$	0.8954
18	rs1057251	32102579	C	T	*MOCOS*	$6.18x10^{-5}$	0.8752
18	rs12457919	32108100	A	C	*MOCOS,FHOD3*	$5.52x10^{-5}$	0.871
19	rs2097982	62472846	A	G	*ZNF805,ZNF460*	$3.35x10^{-5}$	0.6119
20	rs686237	47804141	A	C	*B4GALT5,SLC9A8*	$5.43x10^{-5}$	0.5867
21	rs13046992	40221984	A	G	*PCP4,IGSF5,DSCAM*	$3.81x10^{-5}$	0.9652
21	rs2837308	40229327	A	G	*PCP4,DSCAM*	$4.92x10^{-5}$	0.7549

AJRCCM Articles in Press. Published on 30-November-2016 as 10.1164/rccm.201605-1026OC

Table E4. Top SNPs ($p<1.x10^{-4}$) from the genome-wide interaction meta-analysis of association between SNPxNO_2 and asthma, using the two-step approach.

								Discovery GWIS meta-analysis	**Look-up**	
								n=1,534	**CHS n=1,602**	**CAPPS/ SAGE n=186**
Chr	**SNP**	**Position (build 37)**	**Minor Allele**	**Major Allele**	**MAF**	**Nearest gene**	**Feature**	**p-value***	**p-value†**	**p-value†**
3	rs7651862	65,094,910	T	G	0.48	*MAGI1*	-	$1.87x10^{-7}$	0.77	NA
3	rs11706125	65,094,803	A	G	0.48	*MAGI1*	-	$3.33x10^{-7}$	NA	NA
3	rs11718057	65,094,783	T	A	0.48	*MAGI1*	-	$3.59x10^{-7}$	0.77	NA
3	rs13066946	65,095,948	A	G	0.48	*MAGI1*	-	$3.77x10^{-7}$	0.76	NA
18	rs12457919	33,854,102	C	A	0.10	*MOCOS,FHOD3*	-	$5.52x10^{-5}$	0.017	NA
18	rs12455842	33,842,286	C	T	0.10	*MOCOS, ELP2, RNU4P3*	intron	$6.10x10^{-5}$	0.014	0.55
18	rs1057251	33,848,581	T	C	0.10	*MOCOS, RNU4P3, LOC791126*	missense	$6.18x10^{-5}$	0.013	0.58

Shown are top SNPs from the discovery phase, using the two-step approach, ordered by p-value. All p-values given are two-sided. Chr, chromosome; Minor Allele, according to discovery phase cohorts; MAF, minor allele frequency according to BAMSE; NA, SNP not available. *Genome-wide significance threshold $p<4.18x10^{-7}$, after Bonferroni correction of 119.521 SNPs that were tested in the second step of the two-step approach. †Significance threshold , $p<0.05$.

Table E5. Top SNPs ($p<1.x10^{-4}$) from the genome-wide interaction meta-analysis of association between SNPxNO_2 and asthma, using the 2 df test

Online Repository - GWIS traffic air pollution and asthma

SNP	CHR	POS	Meta-analysis p-value	p-value BAMSE	p-value PIAMA	p-value GINI/LISA	Gene	ensembl_gene_id
rs3825270	12	13115294	1.08X10-6	9.13X10-6	5.10X10-3	1.15X10-1	KIAA1467	ENSG00000084444
rs12424184	12	13113046	1.98X10-6	1.02X10-5	5.09X10-3	2.02X10-1	KIAA1467	ENSG00000084444
rs1386004	12	13104726	2.00X10-6	1.01X10-5	5.07X10-3	2.09X10-1	KIAA1467	ENSG00000084444
rs4763925	12	13110340	2.00X10-6	1.03X10-5	5.09X10-3	2.04X10-1	KIAA1467	ENSG00000084444
rs3803097	12	13110639	2.00X10-6	1.03X10-5	5.09X10-3	2.04X10-1	KIAA1467	ENSG00000084444
rs1552389	12	13091145	2.01X10-6	1.00X10-5	4.99X10-3	2.14X10-1	KIAA1467	ENSG00000084444
rs1463623	12	13090515	2.01X10-6	1.00X10-5	4.99X10-3	2.14X10-1	KIAA1467	ENSG00000084444
rs3741818	12	13105804	2.01X10-6	1.01X10-5	5.08X10-3	2.08X10-1	KIAA1467	ENSG00000084444
rs3741817	12	13106058	2.01X10-6	1.01X10-5	5.09X10-3	2.08X10-1	KIAA1467	ENSG00000084444
rs7956640	12	13094384	2.01X10-6	1.00X10-5	5.00X10-3	2.14X10-1	KIAA1467	ENSG00000084444
rs7963271	12	13101279	2.01X10-6	1.00X10-5	5.04X10-3	2.12X10-1	KIAA1467	ENSG00000084444
rs7303584	12	13097367	2.02X10-6	1.00X10-5	5.02X10-3	2.14X10-1	KIAA1467	ENSG00000084444
rs4763923	12	13099175	2.02X10-6	1.00X10-5	5.03X10-3	2.14X10-1	KIAA1467	ENSG00000084444
rs7956609	12	13094309	2.03X10-6	1.00X10-5	5.05X10-3	2.14X10-1	KIAA1467	ENSG00000084444
rs4763920	12	13087757	2.05X10-6	1.03X10-5	5.00X10-3	2.14X10-1	KIAA1467	ENSG00000255621
rs4077753	5	152175101	2.13X10-6	1.55X10-6	7.12X10-1	1.03X10-2	LINC01470	ENSG00000249484
rs4077752	5	152175070	2.14X10-6	1.55X10-6	7.12X10-1	1.04X10-2	LINC01470	ENSG00000249484
rs13168888	5	152171627	2.14X10-6	1.55X10-6	7.12X10-1	1.04X10-2	LINC01470	ENSG00000249484
rs4583879	5	152169871	2.19X10-6	1.55X10-6	7.31X10-1	1.04X10-2	LINC01470	ENSG00000249484
rs4763924	12	13099819	2.20X10-6	7.92X10-6	7.12X10-3	2.10X10-1	KIAA1467	ENSG00000084444

AJRCCM Articles in Press. Published on 30-November-2016 as 10.1164/rccm.201605-1026OC

rs2741043	2	234232340	2.41X10-6	1.25X10-6	1.65X10-1	6.33X10-2	UGT1A10	ENSG00000242366
rs1377460	2	234201376	2.46X10-6	1.23X10-6	1.57X10-1	6.95X10-2	UGT1A8	ENSG00000242366
rs11055218	12	13103493	2.59X10-6	1.00X10-5	6.74X10-3	2.10X10-1	KIAA1467	ENSG00000084444
rs2924450	2	234229144	2.61X10-6	1.36X10-6	1.67X10-1	6.30X10-2	UGT1A10	ENSG00000242366
rs2741031	2	234203455	2.66X10-6	1.26X10-6	1.67X10-1	6.94X10-2	UGT1A8	ENSG00000242366
rs13183376	5	152188573	2.73X10-6	1.73X10-6	6.90X10-1	1.26X10-2	LINC01470	ENSG00000249484
rs11738913	5	152178685	2.76X10-6	1.55X10-6	7.13X10-1	1.38X10-2	LINC01470	ENSG00000249484
rs4763922	12	13092766	3.14X10-6	2.56X10-5	3.31X10-3	2.08X10-1	KIAA1467	ENSG00000084444
rs10492247	12	13091857	3.35X10-6	1.00X10-5	8.81X10-3	2.14X10-1	KIAA1467	ENSG00000084444
rs1817154	2	234225330	3.47X10-6	1.37X10-6	2.27X10-1	6.30X10-2	UGT1A10	ENSG00000242366
rs7670760	4	138936781	4.35X10-6	4.17X10-4	7.70X10-1	7.90X10-5		
rs1552682	10	133920710	5.59X10-6	6.03X10-7	1.62X10-1	3.45X10-1	KIAA1467	ENSG00000165752
rs9804722	12	13107528	6.43X10-6	8.03X10-5	3.08X10-3	1.59X10-1	KIAA1467	ENSG00000084444
rs1316824	12	13118808	7.08X10-6	1.38X10-4	4.40X10-3	7.25X10-2	KIAA1467	ENSG00000084444
rs2175071	4	138913175	7.18X10-6	1.58X10-2	9.31X10-1	3.02X10-6		
rs11737900	5	152168653	9.20X10-6	7.82X10-6	7.56X10-1	9.95X10-3	LINC01470	ENSG00000249484
rs7445606	5	152180302	9.26X10-6	1.08X10-6	7.45X10-1	7.36X10-2	LINC01470	ENSG00000249484
rs10034241	4	138934133	9.33X10-6	1.89X10-2	8.29X10-1	3.83X10-6		
rs1509265	4	138943226	9.35X10-6	1.90X10-2	8.32X10-1	3.80X10-6		
rs1568967	4	138932735	9.43X10-6	1.89X10-2	8.29X10-1	3.87X10-6		
rs1509268	4	138931754	9.44X10-6	1.89X10-2	8.29X10-1	3.87X10-6		
rs999842	15	20551713	1.06X10-5	2.34X10-4	1.38X10-1	2.15X10-3	NIPA2	ENSG00000140157

Online Repository - GWIS traffic air pollution and asthma

rs12415107	10	71983648	1.08X10-5	2.87X10-7	2.99X10-1	8.20X10-1	PALD1	ENSG00000107719
rs3910954	4	138821160	1.15X10-5	4.28X10-2	4.83X10-1	3.68X10-6	LOC101927414	
rs1532896	18	51631900	1.22X10-5	2.14X10-6	1.57X10-1	2.41X10-1	LOC105372130	ENSG00000267172
rs4958581	5	152167922	1.24X10-5	2.40X10-5	6.69X10-1	5.12X10-3	LINC01470	ENSG00000249484
rs12517563	5	152188838	1.41X10-5	1.55X10-6	7.32X10-1	8.40X10-2	LINC01470	ENSG00000249484
rs237191	16	26580307	1.44X10-5	1.54X10-2	5.49X10-4	1.16X10-2		
rs7179062	15	20553025	1.47X10-5	2.83X10-4	2.48X10-1	1.43X10-3	NIPA2	ENSG00000140157
rs17213661	4	138950587	1.55X10-5	4.53X10-2	8.19X10-1	2.86X10-6	RP11-793B23.1	ENSG00000250034
rs9944650	18	51638460	1.80X10-5	3.32X10-6	1.57X10-1	2.41X10-1	LOC105372130	ENSG00000267172
rs7303887	12	13082442	1.82X10-5	1.97X10-4	7.11X10-3	9.10X10-2	RP11-377D9.3	ENSG00000255621
rs9319731	18	51646327	1.89X10-5	3.50X10-6	1.57X10-1	2.41X10-1		
rs1467032	4	138963335	1.95X10-5	4.05X10-2	8.10X10-1	4.19X10-6		
rs11166507	1	101002400	2.21X10-5	1.75X10-4	3.45X10-3	2.64X10-1		
rs11940232	4	138953336	2.23X10-5	4.62X10-2	8.17X10-1	4.24X10-6	RP11-793B23.1	ENSG00000250034
rs2978529	8	62281020	2.33X10-5	4.26X10-3	3.70X10-3	1.07X10-2	CLVS1	ENSG00000177182
rs4763321	12	13133937	2.39X10-5	6.27X10-5	6.97X10-2	3.97X10-2	KIAA1467	ENSG00000084444
rs6680442	1	100996734	2.42X10-5	2.31X10-4	3.74X10-3	2.03X10-1		
rs4614937	2	18731669	2.42X10-5	7.92X10-6	5.87X10-1	3.79X10-2		
rs9464379	6	56311633	2.48X10-5	3.60X10-4	7.69X10-2	6.55X10-3	COL21A1	ENSG00000124749
rs7554373	1	100998523	2.53X10-5	1.88X10-4	3.74X10-3	2.64X10-1		
rs7179447	15	20553224	2.60X10-5	2.96X10-4	3.05X10-1	2.11X10-3	NIPA2	ENSG00000140157
rs7672176	4	138913964	2.67X10-5	5.91X10-3	8.99X10-1	3.71X10-5		

AJRCCM Articles in Press. Published on 30-November-2016 as 10.1164/rccm.201605-1026OC

rs1526240	4	138860824	2.85X10-5	1.76X10-1	6.55X10-1	1.83X10-6	NIPA2	ENSG00000250777
rs6535922	4	138859507	2.86X10-5	1.76X10-1	6.54X10-1	1.84X10-6	NIPA2	ENSG00000250777
rs11151379	18	51665841	3.07X10-5	5.94X10-6	1.58X10-1	2.46X10-1		
rs11151377	18	51662188	3.10X10-5	6.11X10-6	1.57X10-1	2.43X10-1		
rs3849640	4	138839286	3.20X10-5	1.80X10-1	6.79X10-1	1.98X10-6	NIPA2	ENSG00000250777
rs1526241	4	138863957	3.27X10-5	1.77X10-1	6.60X10-1	2.13X10-6	NIPA2	ENSG00000250777
rs1553600	18	51665132	3.29X10-5	6.08X10-6	1.57X10-1	2.61X10-1		
rs1506630	18	51656338	3.30X10-5	6.14X10-6	1.57X10-1	2.60X10-1		
rs3843891	4	138828912	3.32X10-5	2.44X10-2	6.11X10-1	1.69X10-5	NIPA2	ENSG00000250777
rs1526228	4	138834660	3.32X10-5	2.34X10-1	7.60X10-1	1.42X10-6	NIPA2	ENSG00000250777
rs1526226	4	138905805	3.56X10-5	1.90X10-1	6.77X10-1	2.13X10-6		
rs12605156	18	51649112	3.85X10-5	7.87X10-6	1.57X10-1	2.41X10-1		
rs13170178	5	152189295	3.94X10-5	2.84X10-5	7.23X10-1	1.49X10-2	LINC01470	ENSG00000249484
rs9959500	18	51653994	4.02X10-5	8.26X10-6	1.57X10-1	2.42X10-1		
rs11730757	4	138910812	4.05X10-5	1.39X10-1	6.54X10-1	3.48X10-6		
rs3766509	1	145596899	4.07X10-5	8.66X10-4	3.54X10-2	1.04X10-2	ACP6	ENSG00000162836
rs3737843	1	145554345	4.08X10-5	2.09X10-3	2.46X10-2	6.20X10-3	BCL9	ENSG00000116128
rs1505129	18	73982506	4.24X10-5	9.08X10-6	1.44X10-1	2.54X10-1		
rs12454691	18	73979874	4.29X10-5	1.00X10-5	1.38X10-1	2.45X10-1		
rs12457511	18	73981374	4.29X10-5	9.96X10-6	1.38X10-1	2.46X10-1		
rs12526319	6	103172663	4.52X10-5	3.04X10-2	1.42X10-3	8.30X10-3		
rs6699536	1	101049132	4.52X10-5	2.57X10-3	2.86X10-3	4.88X10-2		

Online Repository - GWIS traffic air pollution and asthma

rs7450158	6	103176780	4.54X10-5	3.20X10-2	1.32X10-3	8.51X10-3		
rs12719344	5	125404676	4.55X10-5	1.37X10-4	6.28X10-1	4.21X10-3		
rs9285553	6	103175472	4.59X10-5	3.21X10-2	1.32X10-3	8.60X10-3		
rs11263678	15	20558548	4.72X10-5	4.08X10-4	4.56X10-1	2.03X10-3	NIPA2	ENSG00000140157
rs6571042	6	103217861	4.75X10-5	4.86X10-2	5.73X10-4	1.36X10-2		
rs1890579	6	159779507	4.87X10-5	1.56X10-1	1.14X10-4	2.20X10-2		
rs17039614	2	2345564	4.96X10-5	8.83X10-4	8.45X10-1	5.35X10-4		
rs4629584	5	152162278	4.97X10-5	2.23X10-5	4.51X10-1	3.98X10-2	LINC01470	ENSG00000249484
rs11743031	5	152195623	4.97X10-5	2.84X10-5	6.55X10-1	2.15X10-2	LINC01470	ENSG00000249484
rs4512127	5	152192652	4.99X10-5	2.84X10-5	6.55X10-1	2.16X10-2	LINC01470	ENSG00000249484
rs2978521	8	62279233	5.04X10-5	4.38X10-3	6.80X10-3	1.36X10-2	CLVS1	ENSG00000177182
rs6571045	6	103234957	5.11X10-5	8.63X10-2	7.24X10-4	6.60X10-3		
rs7455007	6	103238946	5.13X10-5	8.58X10-2	7.24X10-4	6.67X10-3		
rs9377379	6	103233198	5.14X10-5	8.67X10-2	7.24X10-4	6.62X10-3		
rs7450897	6	103230477	5.15X10-5	8.70X10-2	7.24X10-4	6.61X10-3		
rs6571044	6	103234938	5.15X10-5	8.64X10-2	7.24X10-4	6.66X10-3		
rs4460127	5	152163941	5.20X10-5	2.18X10-5	8.35X10-1	2.31X10-2	LINC01470	ENSG00000249484
rs11617728	13	24882356	5.23X10-5	4.77X10-5	1.18X10-2	7.54X10-1	ATP8A2	ENSG00000132932
rs1396672	4	138923473	5.33X10-5	1.38X10-1	8.09X10-1	3.88X10-6		
rs7449573	6	103189846	5.40X10-5	7.78X10-2	7.82X10-4	7.23X10-3		
rs2931331	8	62286060	5.45X10-5	2.53X10-3	6.39X10-3	2.75X10-2	CLVS1	ENSG00000177182
rs3942245	2	2350331	5.51X10-5	4.33X10-4	6.17X10-1	1.69X10-3		

AJRCCM Articles in Press. Published on 30-November-2016 as 10.1164/rccm.201605-1026OC

rs2978535	8	62281668	5.63X10-5	2.54X10-3	6.75X10-3	2.69X10-2		ENSG00000177182
rs7651862	3	65069950	5.64X10-5	2.36X10-2	1.10X10-1	1.78X10-4		
rs4524565	5	152241237	5.65X10-5	1.18X10-5	6.88X10-1	5.69X10-2	LINC01470	ENSG00000249484
rs7451370	6	103186935	5.71X10-5	5.95X10-2	7.95X10-4	9.90X10-3		
rs9404226	6	103204373	5.83X10-5	8.80X10-2	7.60X10-4	7.19X10-3		
rs7746320	6	103194912	5.96X10-5	8.81X10-2	7.76X10-4	7.20X10-3		
rs1051992	11	6297282	6.07X10-5	3.90X10-4	4.21X10-2	3.07X10-2	PRKCDBP	ENSG00000170955
rs2241006	12	9639476	6.11X10-5	8.48X10-2	3.41X10-4	1.75X10-2	KLRB1	ENSG00000111796
rs1537720	9	116937364	6.13X10-5	6.72X10-5	9.27X10-2	8.16X10-2	RP11-532L1.2	ENSG00000236461
rs2676622	9	114494325	6.26X10-5	4.58X10-5	1.33X10-2	8.56X10-1	INIP	ENSG00000148153
rs9511787	13	24881592	6.30X10-5	1.67X10-4	9.10X10-3	3.45X10-1	ATP8A2	ENSG00000132932
rs6903735	6	103254295	6.32X10-5	6.87X10-2	1.40X10-3	5.48X10-3		
rs12052006	18	51696461	6.46X10-5	5.71X10-6	1.73X10-1	5.46X10-1	LIC01416	ENSG00000260930
rs1571046	1	101071804	6.52X10-5	2.65X10-3	4.41X10-3	4.67X10-2		
rs9511790	13	24887752	6.53X10-5	1.21X10-4	4.84X10-3	9.36X10-1	ATP8A2	ENSG00000132932
rs9319747	18	51700096	6.65X10-5	5.87X10-6	1.92X10-1	4.95X10-1	LIC01416	ENSG00000260930
rs11962340	6	103299445	6.76X10-5	6.42X10-2	1.95X10-3	4.56X10-3		
rs2978505	8	62261719	6.90X10-5	7.67X10-3	3.59X10-3	2.11X10-2	CLVS1	ENSG00000177182
rs10844140	12	9638555	6.95X10-5	8.60X10-2	2.68X10-4	2.55X10-2	KLRB1	ENSG00000111796
rs6577209	1	101027668	7.09X10-5	1.89X10-3	4.50X10-3	7.09X10-2		
rs11740092	5	152165624	7.31X10-5	3.35X10-5	8.37X10-1	2.22X10-2	LINC01470	ENSG00000249484
rs12867463	13	24881768	7.48X10-5	1.67X10-4	8.68X10-3	4.42X10-1	ATP8A2	ENSG00000132932

Online Repository - GWIS traffic air pollution and asthma

rs11846469	14	39750884	7.49X10-5	7.25X10-2	2.15X10-3	4.11X10-3		
rs12889928	14	39751650	7.51X10-5	7.25X10-2	2.16X10-3	4.10X10-3		
rs4129242	6	103192235	7.62X10-5	8.83X10-2	1.04X10-3	7.13X10-3		
rs4907399	8	142624890	7.78X10-5	2.71X10-2	4.65X10-2	5.30X10-4		
rs10810259	9	14821864	7.80X10-5	2.80X10-4	1.62X10-1	1.48X10-2	FREM1	ENSG00000164946
rs17214448	4	76702005	7.92X10-5	1.99X10-5	3.99X10-1	8.58X10-2	C4orf26	ENSG00000174792
rs9511786	13	24881275	7.98X10-5	1.68X10-4	9.29X10-3	4.42X10-1	ATP8A2	ENSG00000132932
rs9456749	6	162518420	8.04X10-5	1.37X10-3	1.23X10-1	4.11X10-3	PARK2	ENSG00000185345
rs11734102	4	76701191	8.08X10-5	1.98X10-5	4.02X10-1	8.79X10-2	C4orf26	ENSG00000174792
rs6811713	4	76701395	8.13X10-5	1.99X10-5	4.06X10-1	8.72X10-2	C4orf26	ENSG00000174792
rs1414137	9	116945474	8.27X10-5	7.10X10-5	1.42X10-1	7.13X10-2	RP11-532L1.2	ENSG00000236461
rs13167390	5	152162761	8.37X10-5	2.21X10-5	8.26X10-1	3.98X10-2	LINC01470	ENSG00000249484
rs1135816	12	9641936	8.46X10-5	1.07X10-1	3.77X10-4	1.83X10-2	KLRB1	ENSG00000111796
rs10518644	1	80652485	8.55X10-5	1.73X10-2	1.19X10-2	3.61X10-3		
rs1994519	11	6298324	8.60X10-5	5.30X10-4	4.56X10-2	3.11X10-2	PRKCDBP	ENSG00000170955
rs4736874	8	40576152	8.61X10-5	1.66X10-2	3.14X10-1	1.44X10-4	ZMAT4	ENSG00000165061
rs7216020	17	30121041	8.62X10-5	2.66X10-2	3.22X10-2	8.80X10-4		
rs1523415	6	103264532	8.66X10-5	7.73X10-2	1.98X10-3	4.93X10-3		
rs1523408	6	103268815	8.72X10-5	7.73X10-2	1.98X10-3	4.97X10-3		
rs10743737	12	9514883	8.82X10-5	1.09X10-1	1.25X10-4	5.67X10-2	RP11-726G1.1	ENSG00000214776
rs2051090	13	35352193	8.83X10-5	4.25X10-2	2.99X10-1	6.09X10-5	DCLK1	ENSG00000133083
rs7248327	19	13497913	8.90X10-5	3.84X10-2	1.98X10-3	1.03X10-2	CACNA1A	ENSG00000141837

AJRCCM Articles in Press. Published on 30-November-2016 as 10.1164/rccm.201605-1026OC

rs8028189	15	20575442	8.92X10-5	5.76X10-4	5.53X10-1	2.46X10-3	NIPA2	ENSG00000140157
rs10102149	8	104883444	8.92X10-5	1.08X10-1	3.81X10-3	1.91X10-3	RIMS2	ENSG00000176406
rs3809572	15	65143828	8.97X10-5	3.91X10-5	1.83X10-1	1.10X10-1	RP11-798K3.2	ENSG00000259347
rs10834971	11	26337381	9.04X10-5	9.63X10-4	9.92X10-2	8.32X10-3	ANO3	ENSG00000134343
rs1008560	22	20887954	9.22X10-5	1.33X10-1	1.51X10-2	4.04X10-4	IGLVI-56	ENSG00000253126
rs7170784	15	20572554	9.27X10-5	5.18X10-4	5.80X10-1	2.72X10-3	NIPA2	ENSG00000140157
rs1823803	2	234203850	9.31X10-5	3.74X10-4	3.40X10-2	6.48X10-2	UGT1A8	ENSG00000242366
rs12300846	12	9634751	9.48X10-5	4.32X10-2	5.83X10-4	3.34X10-2	KLRB1	ENSG00000111796
rs11638002	15	65129033	9.53X10-5	3.90X10-5	1.85X10-1	1.17X10-1	RP11-798K3.2	ENSG00000259347
rs1936002	13	35354084	9.68X10-5	4.28X10-2	2.99X10-1	6.73X10-5	DCLK1	ENSG00000133083
rs3000528	10	133922016	9.71X10-5	1.18X10-5	2.33X10-1	3.14X10-1	STK32C	ENSG00000165752
rs2685501	17	53256676	9.71X10-5	1.28X10-3	7.74X10-1	8.72X10-4	RP11-60A24.3	ENSG00000265542
rs9545602	13	35354503	9.72X10-5	4.29X10-2	2.99X10-1	6.75X10-5	DCLK1	ENSG00000133083
rs4734731	8	104904087	9.79X10-5	0.108	3.80X10-3	2.12X10-3	RIMS2	ENSG00000176406
rs9545604	13	35354942	9.79X10-5	4.32X10-2	2.99X10-1	6.75X10-5	DCLK1	ENSG00000133083
rs4943350	13	35351860	9.91X10-5	4.39X10-2	0.299	6.74X10-5	DCLK1	ENSG00000133083

Online Repository - GWIS traffic air pollution and asthma

Table E6. Two df test results in the discovery cohorts for the four SNPs close to the *MAGI1* gene and the eight SNPs with p<0.05 in CHS.

SNP	**CHR**	**POS**	**Meta-analysis p-value**	**p-value BAMSE**	**p-value PIAMA**	**p-value GINI/LISA**	**Gene**
rs7651862	3	65069950	$5{,}64\text{x}10^{-5}$	0,02	0,11	$1{,}78\text{x}10^{-4}$	*MAGI1*
rs11706125	3	65069843	$1{,}11\text{x}10^{-4}$	0,02	0,11	$3{,}89\text{x}10^{-4}$	*MAGI1*
rs11718057	3	65069823	$1{,}21\text{x}10^{-4}$	0,02	0,11	$4{,}29\text{x}10^{-4}$	*MAGI1*
rs13066946	3	65070988	$1{,}24\text{x}10^{-4}$	0,02	0,11	$4{,}32\text{x}10^{-4}$	*MAGI1*
rs686237	20	47804141	0,005	0,006	0,261	0,049	*B4GALT5,*
rs1057251	18	32102579	0,004	0,034	0,062	0,033	*MOCOS*
rs12455842	18	32096284	0,004	0,032	0,074	0,032	*MOCOS*
rs4143882	5	7717364	0,006	0,056	0,083	0,027	*ADCY2*
rs727432	5	7716078	0,008	0,073	0,088	0,027	*ADCY2*
rs6886921	5	7718539	0,001	0,027	0,079	0,009	*ADCY2*
rs12457919	18	32108100	0,004	0,033	0,061	0,034	*MOCOS*
rs963146	11	83423444	0,002	0,039	0,035	0,021	*DLG2*

AJRCCM Articles in Press. Published on 30-November-2016 as 10.1164/rccm.201605-1026OC

Online Repository - GWIS traffic air pollution and asthma

Table E7. Statistically significant SNPs in CAPPS/SAGE from the genome-wide interaction analysis of the association between SNPxNO_2 interaction and asthma.

								Discovery GWIS meta-analysis	**Look-up**	
								n=1,534	**CHS n=1,602**	**CAPPS/SAGE n=186**
Chr	**SNP**	**Position (build 37)**	**Minor Allele**	**Major Allele**	**MAF**	**Nearest gene**	**Feature**	**p-value***	**p-value†**	**p-value†**
4	rs3843891	138828912	C	A	0.47	*PCDH18, SLC7A11*	-	2.49×10^{-5}	0.67	0.037
8	rs17265947	63901079	G	A	0.17	*NKAIN3*	Intron	4.65×10^{-5}	0.30	0.044

Shown are SNPs with p<0.05 in CAPPS/SAGE, ordered by p-value. All p-values given are two-sided. Chr, chromosome; Minor Allele, according to discovery phase cohorts; MAF, minor allele frequency according to BAMSE. *Genome-wide significance threshold, $p<7.2 \times 10^{-8}$. †Significance threshold, p<0.05.

Online Repository - GWIS traffic air pollution and asthma

Table E8. Main effects of SNPs that were statistically significant (p<0.05) in the look-up evaluation in CHS, showing beta coefficients and standard errors.

		Discovery GWIS meta-analysis			**Look-up**					
		BAMSE, GINI/LISA, PIAMA, n=1,534			**CHS n=1,602**			**CAPPS/SAGE n=186**		
Chr	**SNP**	**Combined coef (direction)***	**Combined se***	**Combined p-value†**	**coef**	**se**	**p-value**	**coef**	**se**	**p-value**
20	rs686237	-0.096 (---)	0.104	0.272	-0.016	0.081	0.847	NA	NA	NA
18	rs1057251	-0.104 (--+)	0.160	0.414	-0.062	0.117	0.598	0.022	0.333	0.948
18	rs12455842	-0.133 (--+)	0.161	0.344	-0.069	0.118	0.560	0.059	0.329	0.857
5	rs4143882	-0.015 (--+)	0.103	0.619	0.030	0.078	0.697	0.017	0.196	0.932
5	rs727432	0.009 (++-)	0.103	0.651	-0.027	0.077	0.728	-0.016	0.196	0.937
5	rs6886921	0.013 (++-)	0.102	0.612	-0.044	0.078	0.576	NA	NA	NA
18	rs12457919	0.107 (++-)	0.163	0.419	0.069	0.123	0.573	NA	NA	NA
11	rs963146	0.146 (+-+)	0.113	0.284	-0.050	0.091	0.581	0.097	0.229	0.673

Chr, chromosome; NA, not available. *Metal meta-analysis based on standard errors and estimated effect size for each marker. This analysis assumes consistent effect sizes across studies and was done in order to obtain combined coefficient and combined standard errors for the discovery cohorts. †Metal meta-analysis based on direction of effect for tested allele and corresponding p-value using sample size weighted analysis.

AJRCCM Articles in Press. Published on 30-November-2016 as 10.1164/rccm.201605-1026OC

Table E9. Interaction effects of SNPs that were statistically significant ($p<0.05$) in the look-up evaluation in CHS, showing beta coefficients and standard errors.

		Discovery GWIS meta-analysis			**Look-up**					
		BAMSE, GINI/LISA, PIAMA, n=1,534			**CHS, n=1,602**			**CAPPS/SAGE, n=186**		
Chr	**SNP**	**Combined coef (direction)***	**Combined se***	**Combined p-value†**	**coef**	**se**	**p-value**	**coef**	**se**	**p-value**
20	rs686237	0.683 (+++)	0.170	$5.43x10^{-5}$	-0,253	0,080	0.0016	NA	NA	NA
18	rs1057251	-1.210 (---)	0.324	$6.18x10^{-5}$	0,310	0,119	0.0094	-0.545	0.987	0.581
18	rs12455842	-1.215 (---)	0.327	$6.10x10^{-5}$	0,306	0,119	0,01	-0.587	0.983	0.550
5	rs4143882	0.699 (+++)	0.174	$4.75x10^{-5}$	0,185	0,076	0,015	-0.569	0.510	0.264
5	rs727432	-0.682 (---)	0.174	$6.67x10^{-5}$	-0.183	0,076	0,016	0.564	0.509	0.268
5	rs6886921	-0.769 (---)	0.177	$7.03x10^{-6}$	-0.183	0,076	0,016	NA	NA	NA
18	rs12457919	1.246 (+++)	0.329	$5.52x10^{-5}$	-0.312	0,124	0,012	NA	NA	NA
11	rs963146	0.757 (+++)	0.209	$8.61x10^{-5}$	-0.186	0,088	0,034	-0.227	0.457	0.619

Chr, chromosome; NA, not available. *Metal meta-analysis based on standard errors and estimated effect size for each marker. This analysis assumes consistent effect sizes across studies and was done in order to obtain combined coefficient and combined standard errors for the discovery cohorts. †Metal meta-analysis based on direction of effect for tested allele and corresponding p-value using sample size weighted analysis.

Online Repository - GWIS traffic air pollution and asthma

Table E10. cis-eQTL analysis of association between the 8 top SNPs and expression of genes located within 500k base-pairs of the particular SNP in lung-tissue from 1,111 adults who underwent lung surgery at three academic sites.

Chr	HUGO Gene Symbol*	SNP	SNP Position	Ref. A1	Ref. A2	Affymetrix ID CIS-genes	Chr Start, Affy array	Chr end, Affy array	beta GRO	se GRO	P GRO	Beta LAV	Se LAV	P LAV	Beta UBC	Se UBC	P UBC	Beta meta-analysis	Se meta-analysis	P meta-analysis
5	*C5orf49*	rs727432	7716078	T	G	100307940_TGI_at	7884637	7904173	0.15	0.11	0.16	0.10	0.14	0.47	0.33	0.13	0.01	0.19	0.07	0.01
5	*C5orf49*	rs727432	7716078	T	G	100154445_TGI_at	7884510	7904264	0.11	0.13	0.41	0.16	0.15	0.29	0.40	0.14	0.01	0.21	0.08	0.01
5	*C5orf49*	rs727432	7716078	T	G	100300265_TGI_at	7883750	7904603	0.16	0.10	0.10	0.11	0.12	0.33	0.17	0.12	0.15	0.15	0.06	0.02
5	*ADCY2*	rs727432	7716078	T	G	100138993_TGI_at	7449383	7880644	0.15	0.09	0.09	0.10	0.10	0.33	0.07	0.11	0.54	0.11	0.06	0.05
5	*MTRR*	rs727432	7716078	T	G	100308573_TGI_at	7950283	7953642	-0.07	0.10	0.49	-0.05	0.13	0.69	-0.16	0.10	0.11	-0.10	0.06	0.11
5	*FASTKD3*	rs727432	7716078	T	G	100157808_TGI_at	7912272	7922115	0.12	0.13	0.38	0.28	0.16	0.08	-0.04	0.22	0.86	0.15	0.09	0.11
5	*MTRR*	rs727432	7716078	T	G	100141139_TGI_at	7922216	7954233	-0.11	0.14	0.43	0.33	0.15	0.03	0.01	0.18	0.96	0.07	0.09	0.43
5		rs727432	7716078	T	G	100123278_TGI_at	7556919	7557406	-0.04	0.12	0.75	0.04	0.12	0.76	-0.20	0.16	0.22	-0.04	0.08	0.56
5	*ADCY2*	rs727432	7716078	T	G	100128975_TGI_at	7449342	7883194	-0.03	0.13	0.80	0.02	0.10	0.87	-0.04	0.17	0.82	-0.01	0.07	0.90
5	*C5orf49*	rs4143882	7717364	A	G	100154445_TGI_at	7884510	7904264	0.06	0.10	0.58	0.11	0.10	0.30	0.25	0.11	0.02	0.13	0.06	0.03
5	*C5orf49*	rs4143882	7717364	A	G	100307940_TGI_at	7884637	7904173	0.08	0.08	0.32	0.06	0.10	0.52	0.20	0.10	0.04	0.11	0.05	0.03
5	*MTRR*	rs4143882	7717364	A	G	100308573_TGI_at	7950283	7953642	-0.08	0.08	0.32	-0.10	0.09	0.24	-0.08	0.08	0.27	-0.09	0.05	0.06
5	*FASTKD3*	rs4143882	7717364	A	G	100157808_TGI_at	7912272	7922115	0.07	0.11	0.51	0.18	0.11	0.09	0.10	0.17	0.57	0.12	0.07	0.08
5	*C5orf49*	rs4143882	7717364	A	G	100300265_TGI_at	7883750	7904603	0.10	0.08	0.22	0.08	0.08	0.30	0.06	0.09	0.50	0.08	0.05	0.09
5	*ADCY2*	rs4143882	7717364	A	G	100138993_TGI_at	7449383	7880644	0.12	0.07	0.08	0.03	0.07	0.64	0.02	0.08	0.82	0.06	0.04	0.14
5		rs4143882	7717364	A	G	100123278_TGI_at	7556919	7557406	0.04	0.10	0.69	0.07	0.08	0.37	-0.09	0.12	0.47	0.03	0.06	0.61
5	*MTRR*	rs4143882	7717364	A	G	100141139_TGI_at	7922216	7954233	-0.22	0.11	0.05	0.23	0.10	0.03	-0.07	0.13	0.58	-0.01	0.07	0.93
5	*ADCY2*	rs4143882	7717364	A	G	100128975_TGI_at	7449342	7883194	-0.03	0.10	0.78	-0.00172	0.07	0.98	0.07	0.13	0.57	0.00351	0.05	0.95
5	*C5orf49*	rs6886921	7718539	T	C	100307940_TGI_at	7884637	7904173	0.12	0.10	0.26	0.08	0.13	0.55	0.31	0.12	0.01	0.17	0.07	0.01
5	*C5orf49*	rs6886921	7718539	T	C	100300265_TGI_at	7883750	7904603	0.14	0.10	0.15	0.15	0.11	0.16	0.15	0.11	0.18	0.15	0.06	0.02
5	*C5orf49*	rs6886921	7718539	T	C	100154445_TGI_at	7884510	7904264	0.08	0.13	0.54	0.10	0.14	0.45	0.38	0.14	0.01	0.18	0.08	0.02
5	*ADCY2*	rs6886921	7718539	T	C	100138993_TGI_at	7449383	7880644	0.16	0.09	0.06	0.10	0.10	0.32	0.08	0.11	0.48	0.12	0.06	0.03
5	*FASTKD3*	rs6886921	7718539	T	C	100157808_TGI_at	7912272	7922115	0.14	0.13	0.30	0.27	0.15	0.06	-0.03	0.22	0.91	0.16	0.09	0.07
5	*MTRR*	rs688692	771853	T	C	100308573_TG	795028	795364	-0.06	0.09	0.56	-	0.1	0.98	-0.16	0.1	0.10	-0.08	0.06	0.17

AJRCCM Articles in Press. Published on 30-November-2016 as 10.1164/rccm.201605-1026OC

Online Repository - GWIS traffic air pollution and asthma

		1	9			I_at	3	2				0.00335	2			0				
5		rs6886921	7718539	T	C	100123278_TGI_at	7556919	7557406	-0.02	0.12	0.88	0.01	0.11	0.92	-0.20	0.16	0.20	-0.04	0.07	0.54
5	*ADCY2*	rs6886921	7718539	T	C	100128975_TGI_at	7449342	7883194	-0.07	0.13	0.60	-0.01	0.09	0.93	-0.04	0.17	0.83	-0.03	0.07	0.66
5	*MTRR*	rs6886921	7718539	T	C	100141139_TGI_at	7922216	7954233	-0.13	0.13	0.32	0.23	0.14	0.10	0.00118	0.18	0.99	0.03	0.09	0.72
11	*DLG2*	rs963146	83423444	A	G	100304239_TGI_at	82848509	83705836	0.08	0.10	0.42	0.04	0.09	0.66	0.04	0.11	0.72	0.05	0.06	0.35
11	*DLG2*	rs963146	83423444	A	G	100155681_TGI_at	82843700	84312113	0.05	0.07	0.55	-0.20	0.09	0.02	0.01	0.08	0.90	-0.03	0.05	0.46
18	*SLC39A6*	rs12455842	32096284	T	C	100303106_TGI_at	31944960	31963355	0.21	0.07	0.00271	0.11	0.11	0.33	0.05	0.12	0.67	0.16	0.05	**0.0030**†
18	*RPRD1A*	rs12455842	32096284	T	C	100145446_TGI_at	31859559	31901517	0.07	0.06	0.26	0.18	0.10	0.08	0.03	0.08	0.66	0.08	0.04	0.07
18	*FHOD3*	rs12455842	32096284	T	C	100132210_TGI_at	32131699	32614016	0.02	0.04	0.62	0.20	0.08	0.02	0.04	0.06	0.57	0.05	0.03	0.12
18	*MOCOS*	rs12455842	32096284	T	C	100138664_TGI_at	32021477	32102683	0.00415	0.02	0.86	-0.04	0.03	0.19	-0.05	0.03	0.13	-0.02	0.02	0.18
18	*SLC39A6*	rs12455842	32096284	T	C	100149921_TGI_at	31943196	31963203	-0.01	0.08	0.90	0.23	0.16	0.15	0.20	0.13	0.14	0.08	0.06	0.24
18	*RPRD1A*	rs12455842	32096284	T	C	100158423_TGI_at	31823789	31901371	0.06	0.11	0.59	0.08	0.19	0.68	0.00372	0.16	0.98	0.05	0.08	0.55
18	*ELP2*	rs12455842	32096284	T	C	100140463_TGI_at	31963884	32008605	-0.08	0.10	0.44	0.24	0.19	0.23	0.21	0.15	0.18	0.04	0.08	0.57
18	*RPRD1A*	rs12455842	32096284	T	C	100302024_TGI_at	31823789	31901371	-0.02	0.11	0.85	-0.33	0.22	0.13	0.08	0.18	0.67	-0.05	0.09	0.59
18		rs12455842	32096284	T	C	100146554_TGI_at	32103934	32106109	0.01	0.04	0.75	0.05	0.06	0.44	-0.05	0.06	0.41	0.01	0.03	0.83
18	*SLC39A6*	rs12455842	32096284	T	C	100129017_TGI_at	31943128	31948090	0.00230	0.08	0.98	0.15	0.17	0.38	-0.08	0.13	0.56	0.00427	0.06	0.95
18	*RPRD1A*	rs12455842	32096284	T	C	100136421_TGI_at	31825808	31901520	0.04	0.06	0.57	0.01	0.11	0.94	-0.15	0.12	0.20	-0.00312	0.05	0.95
18		rs12455842	32096284	T	C	100138044_TGI_at	32009221	32011888	-0.06	0.08	0.46	0.01	0.16	0.95	0.15	0.14	0.27	-0.00201	0.06	0.98
18	*FHOD3*	rs1057251	32102579	T	C	100132210_TGI_at	32131699	32614016	0.06	0.06	0.32	0.21	0.09	0.02	0.04	0.08	0.66	0.09	0.04	0.03
18	*MOCOS*	rs1057251	32102579	T	C	100138664_TGI_at	32021477	32102683	-0.03	0.03	0.36	-0.03	0.03	0.28	-0.06	0.04	0.13	-0.04	0.02	0.05
18	*SLC39A6*	rs1057251	32102579	T	C	100303106_TGI_at	31944960	31963355	0.15	0.10	0.13	0.10	0.12	0.38	0.09	0.15	0.54	0.12	0.07	0.07
18	*SLC39A6*	rs1057251	32102579	T	C	100149921_TGI_at	31943196	31963203	0.03	0.12	0.82	0.27	0.16	0.10	0.25	0.17	0.16	0.14	0.08	0.09
18	*SLC39A6*	rs1057251	32102579	T	C	100129017_TGI_at	31943128	31948090	0.23	0.12	0.05	0.15	0.17	0.38	-0.11	0.17	0.53	0.13	0.08	0.13
18	*RPRD1A*	rs1057251	32102579	T	C	100145446_TGI_at	31859559	31901517	0.04	0.09	0.64	0.18	0.11	0.08	0.02	0.10	0.81	0.08	0.06	0.17
18	*RPRD1A*	rs1057251	32102579	T	C	100158423_TGI_at	31823789	31901371	0.19	0.15	0.21	0.11	0.19	0.56	-0.03	0.21	0.88	0.11	0.10	0.28
18	*RPRD1A*	rs1057251	32102579	T	C	100136421_TGI_at	31825808	31901520	0.15	0.09	0.10	-0.01	0.11	0.95	-0.17	0.15	0.27	0.04	0.06	0.51
18		rs1057251	32102579	T	C	100138044_TGI_at	32009221	32011888	-0.00261	0.12	0.98	-0.03	0.16	0.86	0.19	0.18	0.28	0.04	0.08	0.68

Online Repository - GWIS traffic air pollution and asthma

18		rs1057251	32102579	T	C	100146554_TGI_at	32103934	32106109	0.02	0.06	0.76	0.05	0.06	0.42	-0.06	0.08	0.46	0.01	0.04	0.73
18	*RPRD1A*	rs1057251	32102579	T	C	100302024_TGI_at	31823789	31901371	0.12	0.16	0.45	-0.33	0.22	0.13	0.07	0.24	0.77	-0.01	0.11	0.93
18	*ELP2*	rs1057251	32102579	T	C	100140463_TGI_at	31963884	32008605	-0.26	0.15	0.08	0.25	0.20	0.21	0.21	0.20	0.30	-0.00363	0.10	0.97
18	*FHOD3*	rs12457919	32108100	A	C	100132210_TGI_at	32131699	32614016	0.06	0.06	0.32	0.21	0.09	0.02	0.04	0.08	0.66	0.09	0.04	0.03
18	*MOCOS*	rs12457919	32108100	A	C	100138664_TGI_at	32021477	32102683	-0.03	0.03	0.36	-0.03	0.03	0.28	-0.06	0.04	0.13	-0.04	0.02	0.05
18	*SLC39A6*	rs12457919	32108100	A	C	100303106_TGI_at	31944960	31963355	0.15	0.10	0.13	0.10	0.12	0.38	0.09	0.15	0.54	0.12	0.07	0.07
18	*SLC39A6*	rs12457919	32108100	A	C	100149921_TGI_at	31943196	31963203	0.03	0.12	0.82	0.27	0.16	0.10	0.25	0.17	0.16	0.14	0.08	0.09
18	*SLC39A6*	rs12457919	32108100	A	C	100129017_TGI_at	31943128	31948090	0.23	0.12	0.05	0.15	0.17	0.38	-0.11	0.17	0.53	0.13	0.08	0.13
18	*RPRD1A*	rs12457919	32108100	A	C	100145446_TGI_at	31859559	31901517	0.04	0.09	0.64	0.18	0.11	0.08	0.02	0.10	0.81	0.08	0.06	0.17
18	*RPRD1A*	rs12457919	32108100	A	C	100158423_TGI_at	31823789	31901371	0.19	0.15	0.21	0.11	0.19	0.56	-0.03	0.21	0.88	0.11	0.10	0.28
18	*RPRD1A*	rs12457919	32108100	A	C	100136421_TGI_at	31825808	31901520	0.15	0.09	0.10	-0.01	0.11	0.95	-0.17	0.15	0.27	0.04	0.06	0.51
18		rs12457919	32108100	A	C	100138044_TGI_at	32009221	32011888	-0.00261	0.12	0.98	-0.03	0.16	0.86	0.19	0.18	0.28	0.04	0.08	0.68
18		rs12457919	32108100	A	C	100146554_TGI_at	32103934	32106109	0.02	0.06	0.76	0.05	0.06	0.42	-0.06	0.08	0.46	0.01	0.04	0.73
18	*RPRD1A*	rs12457919	32108100	A	C	100302024_TGI_at	31823789	31901371	0.12	0.16	0.45	-0.33	0.22	0.13	0.07	0.24	0.77	-0.01	0.11	0.93
18	*ELP2*	rs12457919	32108100	A	C	100140463_TGI_at	31963884	32008605	-0.26	0.15	0.08	0.25	0.20	0.21	0.21	0.20	0.30	-0.00363	0.10	0.97
20	*B4GALT5*	rs686237	47804141	C	A	100313047_TGI_at	47683394	47685434	0.28	0.08	2.88x10-4	0.49	0.09	3.45x108	0.51	0.09	1.99x108	0.41	0.05	**1.18x10-17**‡
20	*SLC9A8*	rs686237	47804141	C	A	100308978_TGI_at	47938547	47938876	0.05	0.15	0.74	-0.23	0.14	0.10	-0.36	0.17	0.03	-0.17	0.09	0.05
20	*B4GALT5*	rs686237	47804141	C	A	100302697_TGI_at	47682889	47763828	0.12	0.16	0.45	0.29	0.20	0.14	0.17	0.19	0.36	0.18	0.10	0.07
20		rs686237	47804141	C	A	100162632_TGI_at	47747539	47747881	0.08	0.09	0.36	-0.19	0.10	0.07	-0.18	0.11	0.08	-0.07	0.06	0.19
20	*SPATA2*	rs686237	47804141	C	A	100137358_TGI_at	47954772	47965452	0.07	0.13	0.59	0.05	0.22	0.81	0.35	0.23	0.12	0.12	0.10	0.22
20	*SPATA2*	rs686237	47804141	C	A	100133421_TGI_at	47953337	47965475	-0.00230	0.13	0.99	0.32	0.18	0.08	-0.04	0.19	0.85	0.07	0.09	0.43
20	*SLC9A8*	rs686237	47804141	C	A	100140649_TGI_at	47862656	47942179	0.21	0.15	0.17	-0.28	0.19	0.14	0.15	0.18	0.40	0.06	0.10	0.56
20	*RNF114*	rs686237	47804141	C	A	100155758_TGI_at	47986320	48003827	-0.02	0.19	0.91	0.29	0.33	0.38	0.06	0.26	0.81	0.06	0.14	0.68
20	*PTGIS*	rs686237	47804141	C	A	100143106_TGI_at	47553817	47618114	-0.13	0.09	0.16	0.10	0.12	0.37	0.02	0.11	0.88	-0.02	0.06	0.69
20	*SLC9A8*	rs686237	47804141	C	A	100152866_TGI_at	47927940	47940338	0.18	0.17	0.27	-0.08	0.22	0.73	-0.11	0.24	0.65	0.04	0.12	0.71
20	*SLC9A8*	rs686237	47804141	C	A	100308967_TGI_at	47940839	47941354	-0.0018	0.16	0.99	0.16	0.17	0.36	-0.09	0.21	0.67	0.03	0.10	0.74

AJRCCM Articles in Press. Published on 30-November-2016 as 10.1164/rccm.201605-1026OC

												6							
20	*SI1*	rs686237	47804141	C	A	100127534_TGI_at	48032933	48038825	0.05	0.07	0.48	-0.00250	0.09	0.98	-0.02	0.08	0.82	0.01	0.04	0.74
20		rs686237	47804141	C	A	100146092_TGI_at	47621459	47621878	-0.02	0.06	0.73	0.05	0.08	0.57	-0.05	0.09	0.55	-0.01	0.04	0.82
20	*RNF114*	rs686237	47804141	C	A	100162528_TGI_at	47986356	48002083	-0.06	0.15	0.67	0.16	0.23	0.49	-0.08	0.19	0.70	-0.02	0.11	0.84

Adjusted for age, gender, smoking, and disease (e.g. COPD), principal components explaining > 1% of variance (to remove possible noise/variation due to technical factors). GRO, University of Groningen; LAV, Laval University; UBC, University of British Columbia. *Cis-eQTL analysis of genes located within 500,000 bp of the SNPs (using imputed data). †Significant after correction for multiple testing using the Bonferroni method (0.05/12=0.0042) ‡significant after correction for multiple testing using the Bonferroni method (0.05/14=0.0036)..

Table E11. Cis-eQTL analysis of association between the 8 top SNPs and expression of genes located within 500k base-pairs of the particular SNP in whole blood from the GTEx database.

Chr	Gene Symbol	Gencode Id	SNP	p-value	Effect Size	T-Statistic	Standard Error
20	B4GALT5	ENSG00000158470.5	rs686237	0.0004*	-0.1	-3.6	0.029
5	ADCY2	ENSG00000078295.11	rs6886921	0.00045*	-0.19	-3.5	0.053
5	ADCY2	ENSG00000078295.11	rs727432	0.00048*	-0.19	-3.5	0.054
5	ADCY2	ENSG00000078295.11	rs4143882	0.0012*	-0.18	-3.3	0.055
18	ELP2	ENSG00000134759.9	rs1057251	0.15	-0.098	-1.4	0.068
18	ELP2	ENSG00000134759.9	rs12455842	0.15	-0.098	-1.4	0.068
18	ELP2	ENSG00000134759.9	rs12457919	0.15	-0.098	-1.4	0.068
20	PTGIS	ENSG00000124212.5	rs686237	0.15	0.075	1.5	0.052

Online Repository - GWIS traffic air pollution and asthma

20	SLC9A8	ENSG00000197818.7	rs686237	0.17	-0.04	-1.4	0.029
5	FASTKD3	ENSG00000124279.7	rs6886921	0.22	0.038	1.2	0.031
5	FASTKD3	ENSG00000124279.7	rs727432	0.23	0.038	1.2	0.031
5	MTRR	ENSG00000124275.10	rs727432	0.31	0.036	1	0.035
5	MTRR	ENSG00000124275.10	rs727432	0.31	0.036	1	0.035
5	MTRR	ENSG00000124275.10	rs6886921	0.41	0.029	0.83	0.035
5	MTRR	ENSG00000124275.10	rs4143882	0.42	0.029	0.82	0.036
5	MTRR	ENSG00000124275.10	rs4143882	0.42	0.029	0.82	0.036
5	FASTKD3	ENSG00000124279.7	rs4143882	0.43	0.025	0.79	0.032
18	FHOD3	ENSG00000134775.11	rs1057251	0.56	-0.067	-0.59	0.11
18	FHOD3	ENSG00000134775.11	rs12455842	0.56	-0.066	-0.58	0.11
18	FHOD3	ENSG00000134775.11	rs12457919	0.56	-0.068	-0.59	0.12
20	SPATA2	ENSG00000158480.6	rs686237	0.59	0.017	0.54	0.031
18	SLC39A6	ENSG00000141424.8	rs12457919	0.67	0.029	0.43	0.066
18	SLC39A6	ENSG00000141424.8	rs1057251	0.68	0.028	0.42	0.066

AJRCCM Articles in Press. Published on 30-November-2016 as 10.1164/rccm.201605-1026OC

18	SLC39A6	ENSG00000141424.8	rs12455842	0.68	0.027	0.41	0.066
18	RPRD1A	ENSG00000141425.13	rs12457919	0.74	0.02	0.33	0.061
18	RPRD1A	ENSG00000141425.13	rs1057251	0.75	0.02	0.32	0.061
18	RPRD1A	ENSG00000141425.13	rs12455842	0.75	0.019	0.32	0.061
20	RNF114	ENSG00000124226.7	rs686237	0.81	-0.013	-0.24	0.054
20	RNF114	ENSG00000124226.7	rs686237	0.81	-0.013	-0.24	0.054
5	C5orf49	ENSG00000215217.2 not sufficiently expressed	rs4143882	NA	NA	NA	NA
5	C5orf49	ENSG00000215217.2 not sufficiently expressed	rs6886921	NA	NA	NA	NA
5	C5orf49	ENSG00000215217.2 not sufficiently expressed	rs727432	NA	NA	NA	NA
11	DLG2	ENSG00000150672.12 not sufficiently expressed	rs963146	NA	NA	NA	NA
18	MOCOS	ENSG00000075643.5 not sufficiently expressed	rs1057251	NA	NA	NA	NA
18	MOCOS	ENSG00000075643.5 not sufficiently expressed	rs12455842	NA	NA	NA	NA
18	MOCOS	ENSG00000075643.5 not sufficiently expressed	rs12457919	NA	NA	NA	NA
20	Gene not found	SI1 not found	rs686237	NA	NA	NA	NA

*Significant at the 5% FDR level correction for multiple testing.

Table E12. Association between NO_2 exposure levels at 16 years of age and peripheral blood gene expression levels at 16 years of age in BAMSE (n=243).

Chr	Gene	Probe	Associated SNP	Coef	p-value
5	*ADCY2*	TC05000054.hg.1	rs6886921	0.032	0.021
5	*ADCY2*	TC05000055.hg.1	rs6886921	0.023	0.372
11	*DLG2*	TC11002159.hg.1	rs963146	0.028	0.046
18	*MOCOS*	TC18000149.hg.1	rs1057251	0.041	0.009
20	*B4GALT5*	TC20000928.hg.1	rs686237	-0.043	0.194
20	*SLC9A8*	TC20000391.hg.1	rs686237	-0.023	0.258

Analyses were adjusted for age, sex and cell count. Coef: log fold change in gene expression per 10 µg/m3 increase in NO2 exposure; p-value: p-value for association between NO2 exposure and gene expression.

AJRCCM Articles in Press. Published on 30-November-2016 as 10.1164/rccm.201605-1026OC

Table E13. Associations between SNP and CpG site methylation (cis-methQTL) at 8 yrs in BAMSE (n=460) with lowest ten p-values.

Chr	GWIS locus	SNP	SNP position (build 37)	Probe	Probe position (build 37)	CpG site location	beta	se	p-value
5	*ADCY2*	rs727432[T]	7,663,078	cg02602541	7,850,438	*C5orf49* (Body)	0.0016	0.0006	0.0071
5	*ADCY2*	rs4143882[A]	7,664,364	cg02602541	7,850,438	*C5orf49* (Body)	0.0016	0.0006	0.0085
5	*ADCY2*	rs727432[T]	7,663,078	cg00984474	7,850,922	*C5orf49* (Body)	-0.0015	0.0006	0.014
5	*ADCY2*	rs4143882[A]	7,664,364	cg00984474	7,850,922	*C5orf49* (Body)	-0.0014	0.0006	0.017
5	*ADCY2*	rs6886921[T]	7,718,539	cg02602541	7,850,438	*C5orf49* (Body)	0.0014	0.0006	0.016
11	*DLG2*	rs963146[G]	83,745,796	cg08432013	83,393,570	*DLG2* (Body;TSS200)	-0.0060	0.0021	0.0045
11	*DLG2*	rs963146[G]	83,745,796	cg14716968	84,635,906	*DLG2* (TSS1500;Body)	-0.0089	0.0034	0.0086
20	*B4GALT5*	rs686237[A]	48,370,734	cg12058372	48,252,667	*B4GALT5* (3'UTR)	-0.0060	0.0021	0.0042
20	*B4GALT5*	rs686237[A]	48,370,734	cg27403406	48,325,721	B4GALT5 (Body)	0.0071	0.0028	0.011
20	*B4GALT5*	rs686237[A]	48,370,734	cg02003117	48,428,318	*SLC9A8* (TSS1500)	-0.0033	0.0014	0.021

Adjusted for age at the 8 years follow-up, sex, environmental tobacco smoke exposure during first year of life, NO_2 exposure at birth, municipality, cell type, batch (bisulfite treatment date), and asthma up to 8 years of age. No significant methQTLs were detected at the 5% FDR correction level for multiple testing. Chr, chromosome; SNP, using the major allele as reference with a change in beta for each extra minor allele (minor allele in brackets); Beta, CpG site methylation change per additional minor allele; se, standard error.

Online Repository - GWIS traffic air pollution and asthma

Table E14. Human Protein Atlas protein expression profile of genes in normal respiratory system (lung) and smooth muscle tissue.

		Nasopharynx respiratory epithelial cells		Bronchus respiratory epithelial cells		Lung pneumocytes		Lung macrophages		Smooth muscle tissue	
Chr	GWIS locus	Antibody Staining*	Annotated expression	Antibody staining	Annotated expression	Antibody staining	Annotated expression	Antibody staining	Annotated expression	Antibody staining	Annotated expression
5	*ADCY2*	1-medium, 2-medium, 3-medium,	medium	1-medium, 2-medium, 3-medium	Medium	1-low, 2-medium, 3-low	low	1-medium, 2-medium, 3-high	medium	1-medium, 2-medium, 3-low	medium
11	*DLG2*	1-low	-	1-medium	-	1-ND	-	1-low	-	ND	-
18	*MOCOS*	1-medium, 2-ND	medium	1-medium, 2-low	Medium	1-ND, 2-ND	ND	1-low, 2-ND	low	1-high 2-ND	high
20	*B4GALT5*	NA	-	NA	-	NA	-	NA	-	NA	-

ND, not detected; NA, no antibodies available. *Number of antibodies according to Human Protein Atlas (E38).

AJRCCM Articles in Press. Published on 30-November-2016 as 10.1164/rccm.201605-1026OC

SUPPLEMENTARY REFERENCE LIST

E1. Thacher JD, Gruzieva O, Pershagen G, Neuman A, Wickman M, Kull I, Melen E, Bergstrom A. Pre- and postnatal exposure to parental smoking and allergic disease through adolescence. *Pediatrics* 2014; 134: 428-434.

E2. Moffatt MF, Gut IG, Demenais F, Strachan DP, Bouzigon E, Heath S, von Mutius E, Farrall M, Lathrop M, Cookson WO, consortium. G. A large-scale, consortium-based genomewide association study of asthma. *The New England journal of medicine* 2010; 363: 1211-1221.

E3. Bousquet J, Anto J, Auffray C, Akdis M, Cambon-Thomsen A, Keil T, Haahtela T, Lambrecht BN, Postma DS, Sunyer J, Valenta R, Akdis CA, Annesi-Maesano I, Arno A, Bachert C, Ballester F, Basagana X, Baumgartner U, Bindslev-Jensen C, Brunekreef B, Carlsen KH, Chatzi L, Crameri R, Eveno E, Forastiere F, Garcia-Aymerich J, Guerra S, Hammad H, Heinrich J, Hirsch D, Jacquemin B, Kauffmann F, Kerkhof M, Kogevinas M, Koppelman GH, Kowalski ML, Lau S, Lodrup-Carlsen KC, Lopez-Botet M, Lotvall J, Lupinek C, Maier D, Makela MJ, Martinez FD, Mestres J, Momas I, Nawijn MC, Neubauer A, Oddie S, Palkonen S, Pin I, Pison C, Rance F, Reitamo S, Rial-Sebbag E, Salapatas M, Siroux V, Smagghe D, Torrent M, Toskala E, van Cauwenberge P, van Oosterhout AJ, Varraso R, von Hertzen L, Wickman M, Wijmenga C, Worm M, Wright J, Zuberbier T. MeDALL (Mechanisms of the Development of ALLergy): an integrated approach from phenotypes to systems medicine. *Allergy* 2011; 66: 596-604.

E4. Heinrich J, Bolte G, Holscher B, Douwes J, Lehmann I, Fahlbusch B, Bischof W, Weiss M, Borte M, Wichmann HE, Group LS. Allergens and endotoxin on mothers' mattresses and total immunoglobulin E in cord blood of neonates. *The European respiratory journal* 2002; 20: 617-623.

E5. Berg A, Kramer U, Link E, Bollrath C, Heinrich J, Brockow I, Koletzko S, Grubl A, Filipiak-Pittroff B, Wichmann HE, Bauer CP, Reinhardt D, Berdel D, group GIs. Impact of early feeding on childhood

eczema: development after nutritional intervention compared with the natural course - the GINIplus study up to the age of 6 years. *Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology* 2010; 40: 627-636.

E6. Wijga AH, Kerkhof M, Gehring U, de Jongste JC, Postma DS, Aalberse RC, Wolse AP, Koppelman GH, van Rossem L, Oldenwening M, Brunekreef B, Smit HA. Cohort profile: the prevention and incidence of asthma and mite allergy (PIAMA) birth cohort. *International journal of epidemiology* 2014; 43: 527-535.

E7. Peters JM, Avol E, Navidi W, London SJ, Gauderman WJ, Lurmann F, Linn WS, Margolis H, Rappaport E, Gong H, Thomas DC. A study of twelve Southern California communities with differing levels and types of air pollution. I. Prevalence of respiratory morbidity. *Am J Respir Crit Care Med* 1999; 159: 760-767.

E8. Chan-Yeung M, Manfreda J, Dimich-Ward H, Ferguson A, Watson W, Becker A. A randomized controlled study on the effectiveness of a multifaceted intervention program in the primary prevention of asthma in high-risk infants. *Archives of pediatrics & adolescent medicine* 2000; 154: 657-663.

E9. Carlsten C, Dybuncio A, Becker A, Chan-Yeung M, Brauer M. Traffic-related air pollution and incident asthma in a high-risk birth cohort. *Occupational and environmental medicine* 2011; 68: 291-295.

E10. Kozyrskyj AL, HayGlass KT, Sandford AJ, Pare PD, Chan-Yeung M, Becker AB. A novel study design to investigate the early-life origins of asthma in children (SAGE study). *Allergy* 2009; 64: 1185-1193.

E11. Henderson SB, Beckerman B, Jerrett M, Brauer M. Application of land use regression to estimate long-term concentrations of traffic-related nitrogen oxides and fine particulate matter. *Environmental Science & Technology* 2007; 41: 2422-2428.

E12. Allen RW, Amram O, Wheeler AJ, Brauer M. The transferability of NO and NO2 land use regression models between cities and pollutants. *Atmos Environ* 2011; 45: 369-378.

AJRCCM Articles in Press. Published on 30-November-2016 as 10.1164/rccm.201605-1026OC

E13. McConnell R, Berhane K, Gilliland F, Molitor J, Thomas D, Lurmann F, Avol E, Gauderman WJ, Peters JM. Prospective study of air pollution and bronchitic symptoms in children with asthma. *Am J Respir Crit Care Med* 2003; 168: 790-797.

E14. Gruzieva O, Bellander T, Eneroth K, Kull I, Melen E, Nordling E, van Hage M, Wickman M, Moskalenko V, Hulchiy O, Pershagen G. Traffic-related air pollution and development of allergic sensitization in children during the first 8 years of life. *J Allergy Clin Immunol* 2012; 129: 240-246.

E15. Nordling E, Berglind N, Melen E, Emenius G, Hallberg J, Nyberg F, Pershagen G, Svartengren M, Wickman M, Bellander T. Traffic-related air pollution and childhood respiratory symptoms, function and allergies. *Epidemiology* 2008; 19: 401-408.

E16. Paternoster L, Standl M, Chen CM, Ramasamy A, Bonnelykke K, Duijts L, Ferreira MA, Alves AC, Thyssen JP, Albrecht E, Baurecht H, Feenstra B, Sleiman PM, Hysi P, Warrington NM, Curjuric I, Myhre R, Curtin JA, Groen-Blokhuis MM, Kerkhof M, Saaf A, Franke A, Ellinghaus D, Folster-Holst R, Dermitzakis E, Montgomery SB, Prokisch H, Heim K, Hartikainen AL, Pouta A, Pekkanen J, Blakemore AI, Buxton JL, Kaakinen M, Duffy DL, Madden PA, Heath AC, Montgomery GW, Thompson PJ, Matheson MC, Le Souef P, St Pourcain B, Smith GD, Henderson J, Kemp JP, Timpson NJ, Deloukas P, Ring SM, Wichmann HE, Muller-Nurasyid M, Novak N, Klopp N, Rodriguez E, McArdle W, Linneberg A, Menne T, Nohr EA, Hofman A, Uitterlinden AG, van Duijn CM, Rivadeneira F, de Jongste JC, van der Valk RJ, Wjst M, Jogi R, Geller F, Boyd HA, Murray JC, Kim C, Mentch F, March M, Mangino M, Spector TD, Bataille V, Pennell CE, Holt PG, Sly P, Tiesler CM, Thiering E, Illig T, Imboden M, Nystad W, Simpson A, Hottenga JJ, Postma D, Koppelman GH, Smit HA, Soderhall C, Chawes B, Kreiner-Moller E, Bisgaard H, Melen E, Boomsma DI, Custovic A, Jacobsson B, Probst-Hensch NM, Palmer LJ, Glass D, Hakonarson H, Melbye M, Jarvis DL, Jaddoe VW, Gieger C, Strachan DP, Martin NG, Jarvelin MR, Heinrich J, Evans DM, Weidinger S. Meta-

analysis of genome-wide association studies identifies three new risk loci for atopic dermatitis. *Nat Genet* 2012; 44: 187-192.

E17. Torgerson DG, Ampleford EJ, Chiu GY, Gauderman WJ, Gignoux CR, Graves PE, Himes BE, Levin AM, Mathias RA, Hancock DB, Baurley JW, Eng C, Stern DA, Celedon JC, Rafaels N, Capurso D, Conti DV, Roth LA, Soto-Quiros M, Togias A, Li X, Myers RA, Romieu I, Van Den Berg DJ, Hu D, Hansel NN, Hernandez RD, Israel E, Salam MT, Galanter J, Avila PC, Avila L, Rodriquez-Santana JR, Chapela R, Rodriguez-Cintron W, Diette GB, Adkinson NF, Abel RA, Ross KD, Shi M, Faruque MU, Dunston GM, Watson HR, Mantese VJ, Ezurum SC, Liang L, Ruczinski I, Ford JG, Huntsman S, Chung KF, Vora H, Calhoun WJ, Castro M, Sienra-Monge JJ, del Rio-Navarro B, Deichmann KA, Heinzmann A, Wenzel SE, Busse WW, Gern JE, Lemanske RF, Jr., Beaty TH, Bleecker ER, Raby BA, Meyers DA, London SJ, Gilliland FD, Burchard EG, Martinez FD, Weiss ST, Williams LK, Barnes KC, Ober C, Nicolae DL. Meta-analysis of genome-wide association studies of asthma in ethnically diverse North American populations. *Nat Genet* 2011; 43: 887-892.

E18. Willer CJ, Li Y, Abecasis GR. METAL: fast and efficient meta-analysis of genomewide association scans. *Bioinformatics* 2010; 26: 2190-2191.

E19. R Core Team. R: A Language and Environment for Statistical Computing. Vienna: R Foundation for Statistical Computing; 2013.

E20. Turner SD. qqman: an R package for visualizing GWAS results using Q-Q and manhattan plots. 2014.

E21. Johnson AD, Handsaker RE, Pulit SL, Nizzari MM, O'Donnell CJ, de Bakker PI. SNAP: a web-based tool for identification and annotation of proxy SNPs using HapMap. *Bioinformatics* 2008; 24: 2938-2939.

E22. MacIntyre EA, Brauer M, Melen E, Bauer CP, Bauer M, Berdel D, Bergstrom A, Brunekreef B, Chan-Yeung M, Klumper C, Fuertes E, Gehring U, Gref A, Heinrich J, Herbarth O, Kerkhof M, Koppelman GH, Kozyrskyj AL, Pershagen G, Postma DS, Thiering E, Tiesler CM, Carlsten C, Group

AJRCCM Articles in Press. Published on 30-November-2016 as 10.1164/rccm.201605-1026OC

TAGS. GSTP1 and TNF Gene variants and associations between air pollution and incident childhood asthma: the traffic, asthma and genetics (TAG) study. *Environmental health perspectives* 2014; 122: 418-424.

E23. Murcray CE, Lewinger JP, Conti DV, Thomas DC, Gauderman WJ. Sample size requirements to detect gene-environment interactions in genome-wide association studies. *Genet Epidemiol* 2011; 35: 201-210.

E24. Murcray CE, Lewinger JP, Gauderman WJ. Gene-environment interaction in genome-wide association studies. *Am J Epidemiol* 2009; 169: 219-226.

E25. Kraft P, Yen YC, Stram DO, Morrison J, Gauderman WJ. Exploiting gene-environment interaction to detect genetic associations. *Hum Hered* 2007; 63: 111-119.

E26. Gruzieva O, Xu CJ, Breton CV, Annesi-Maesano I, Anto JM, Auffray C, Ballereau S, Bellander T, Bousquet J, Bustamante M, Charles MA, de Kluizenaar Y, den Dekker HT, Duijts L, Felix JF, Gehring U, Guxens M, Jaddoe VV, Jankipersadsing SA, Merid SK, Kere J, Kumar A, Lemonnier N, Lepeule J, Nystad W, Page CM, Panasevich S, Postma D, Slama R, Sunyer J, Soderhall C, Yao J, London SJ, Pershagen G, Koppelman GH, Melen E. Epigenome-Wide Meta-Analysis of Methylation in Children Related to Prenatal NO2 Air Pollution Exposure. *Environ Health Perspect* 2016.

E27. Schroeder A, Mueller O, Stocker S, Salowsky R, Leiber M, Gassmann M, Lightfoot S, Menzel W, Granzow M, Ragg T. The RIN: an RNA integrity number for assigning integrity values to RNA measurements. *BMC Mol Biol* 2006; 7: 3.

E28. Imbeaud S, Graudens E, Boulanger V, Barlet X, Zaborski P, Eveno E, Mueller O, Schroeder A, Auffray C. Towards standardization of RNA quality assessment using user-independent classifiers of microcapillary electrophoresis traces. *Nucleic Acids Res* 2005; 33: e56.

E29. Affymetrix. Microarray normalization using Signal Space Transformation with probe Guanine Cytosine Count Correction (white paper). 2016.

E30. Irizarry RA, Hobbs B, Collin F, Beazer-Barclay YD, Antonellis KJ, Scherf U, Speed TP. Exploration, normalization, and summaries of high density oligonucleotide array probe level data. *Biostatistics* 2003; 4: 249-264.

E31. Bolstad BM, Irizarry RA, Astrand M, Speed TP. A comparison of normalization methods for high density oligonucleotide array data based on variance and bias. *Bioinformatics* 2003; 19: 185-193.

E32. Johnson WE, Li C, Rabinovic A. Adjusting batch effects in microarray expression data using empirical Bayes methods. *Biostatistics* 2007; 8: 118-127.

E33. Chen YA, Lemire M, Choufani S, Butcher DT, Grafodatskaya D, Zanke BW, Gallinger S, Hudson TJ, Weksberg R. Discovery of cross-reactive probes and polymorphic CpGs in the Illumina Infinium HumanMethylation450 microarray. *Epigenetics* 2013; 8: 203-209.

E34. Pidsley R, CC YW, Volta M, Lunnon K, Mill J, Schalkwyk LC. A data-driven approach to preprocessing Illumina 450K methylation array data. *BMC Genomics* 2013; 14: 293.

E35. Houseman EA, Accomando WP, Koestler DC, Christensen BC, Marsit CJ, Nelson HH, Wiencke JK, Kelsey KT. DNA methylation arrays as surrogate measures of cell mixture distribution. *BMC Bioinformatics* 2012; 13: 86.

E36. Jaffe AE, Irizarry RA. Accounting for cellular heterogeneity is critical in epigenome-wide association studies. *Genome Biol* 2014; 15: R31.

E37. Severin J, Lizio M, Harshbarger J, Kawaji H, Daub CO, Hayashizaki Y, Bertin N, Forrest AR. Interactive visualization and analysis of large-scale sequencing datasets using ZENBU. *Nat Biotechnol* 2014; 32: 217-219.

AJRCCM Articles in Press. Published on 30-November-2016 as 10.1164/rccm.201605-1026OC

E38. Uhlen M, Oksvold P, Fagerberg L, Lundberg E, Jonasson K, Forsberg M, Zwahlen M, Kampf C, Wester K, Hober S, Wernerus H, Bjorling L, Ponten F. Towards a knowledge-based Human Protein Atlas. *Nat Biotechnol* 2010; 28: 1248-1250.