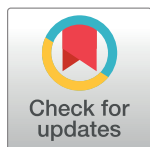


RESEARCH ARTICLE

Adult onset asthma and interaction between genes and active tobacco smoking: The GABRIEL consortium

J. M. Vonk^{1,2,*}, S. Scholtens¹, D. S. Postma^{2,3}, M. F. Moffatt⁴, D. Jarvis^{5,6}, A. Ramasamy⁵, M. Wjst^{7,8}, E. R. Omenaas⁹, E. Bouzigon^{10,11}, F. Demenais^{10,11}, R. Nadif^{12,13}, V. Siroux^{14,15,16}, A. V. Polonikov¹⁷, M. Solodilova¹⁷, V. P. Ivanov¹⁷, I. Curjuric^{18,19}, M. Imboden^{18,19}, A. Kumar^{18,19,20}, N. Probst-Hensch^{18,19}, L. M. Ogorodova²¹, V. P. Puzyrev^{21,22}, E. Yu Bragina²², M. B. Freidin²², I. M. Nolte¹, A. M. Farrall²⁰, W. O. C. M. Cookson⁴, D. P. Strachan²³, G. H. Koppelman^{2,24}, H. M. Boezen^{1,2}



OPEN ACCESS

Citation: Vonk JM, Scholtens S, Postma DS, Moffatt MF, Jarvis D, Ramasamy A, et al. (2017) Adult onset asthma and interaction between genes and active tobacco smoking: The GABRIEL consortium. PLoS ONE 12(3): e0172716. doi:10.1371/journal.pone.0172716

Editor: Yong-Gang Yao, Kunming Institute of Zoology, Chinese Academy of Sciences, CHINA

Received: October 3, 2016

Accepted: February 8, 2017

Published: March 2, 2017

Copyright: © 2017 Vonk et al. This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability Statement: The data from both the GABRIEL consortium and the LifeLines study cannot be made publicly available for ethical and legal reasons. Genome wide SNP data can potentially identify individuals and the written informed consent did not cover consent for making the data publicly available. However, the data are available on request. For the GABRIEL-consortium this request should be submitted to the corresponding author and for LifeLines this request should be submitted to The LifeLines Research Office (email: lifelines@research.nl, Dr. A. Dotinga)

1 University of Groningen, University Medical Center Groningen, Department of Epidemiology, Groningen, the Netherlands, **2** University of Groningen, University Medical Center Groningen, Groningen Research Institute for Asthma and COPD (GRIAC), Groningen, the Netherlands, **3** University of Groningen, University Medical Center Groningen, Department of Pulmonology, Groningen, the Netherlands, **4** Division of Respiratory Sciences, Imperial College, London, United Kingdom, **5** Population Health and Occupational Disease, Imperial College, London, United Kingdom, **6** MRC-PHE Centre for Environment and Health, Imperial College, London, United Kingdom, **7** Institute of Medical Statistics and Epidemiology (IMSE), Klinikum Rechts der Isar, Technical University, Munich, Germany, **8** Comprehensive Pneumology Center (CPC), Institute of Lung Biology and Disease (ILBD), Helmholtz Center Munich, Neuherberg, Germany, **9** Centre for Clinical Research, Haukeland University Hospital, Bergen, Norway, **10** Univ Paris Diderot, Sorbonne Paris Cité, Institut Universitaire d'Hématologie, Paris, France, **11** INSERM, UMR-946, Paris, France, **12** INSERM, U1168, VIMA: Aging and chronic diseases, Epidemiological and public health approaches, Villejuif, France, **13** Univ Versailles St-Quentin-en-Yvelines, UMR-S 1168, Montigny le Bretonneux, France, **14** INSERM, IAB, Team of Environmental Epidemiology applied to Reproduction and Respiratory Health, Grenoble, France, **15** Univ. Grenoble Alpes, IAB, Team of Environmental Epidemiology applied to Reproduction and Respiratory Health, Grenoble, France, **16** CHU de Grenoble, IAB, Team of Environmental Epidemiology applied to Reproduction and Respiratory Health, Grenoble, France, **17** Kursk State Medical University, Department of Biology, Medical Genetics and Ecology, Kursk, Russian Federation, **18** Department of Epidemiology and Public Health, Swiss Tropical and Public Health Institute, Basel, Switzerland, **19** University of Basel, Basel, Switzerland, **20** Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, United Kingdom, **21** Siberian State Medical University, Tomsk, Russia, **22** Research Institute of Medical Genetics, Tomsk NRC, Russia, **23** Population Health Research Institute, St George's, University of London, London, United Kingdom, **24** University of Groningen, University Medical Center Groningen, Department of Pediatric Pulmonology and Pediatric Allergy, Beatrix Children's Hospital, Groningen, the Netherlands

* These authors contributed equally to this work.

* j.m.vonk@umcg.nl

Abstract

Background

Genome-wide association studies have identified novel genetic associations for asthma, but without taking into account the role of active tobacco smoking. This study aimed to identify novel genes that interact with ever active tobacco smoking in adult onset asthma.

Methods

We performed a genome-wide interaction analysis in six studies participating in the GABRIEL consortium following two meta-analyses approaches based on 1) the overall

stating the number and PI of this project: OV10_0065, PI: H.M. Boezen.

Funding: The GABRIEL study (a multidisciplinary study to identify the genetic and environmental causes of asthma in the European Community) was supported by the European Commission, contract number 018996 under the Integrated Program LSH-2004-1.2.5-1. The separate cohort were also supported by local funding organizations (see supplementary material). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: The authors have declared that no competing interests exist.

interaction effect and 2) the genetic effect in subjects with and without smoking exposure. We performed a discovery meta-analysis including 4,057 subjects of European descent and replicated our findings in an independent cohort (LifeLines Cohort Study), including 12,475 subjects.

Results

First approach: 50 SNPs were selected based on an overall interaction effect at $p < 10^{-4}$. The most pronounced interaction effect was observed for rs9969775 on chromosome 9 (discovery meta-analysis: $OR_{int} = 0.50$, $p = 7.63 \times 10^{-5}$, replication: $OR_{int} = 0.65$, $p = 0.02$). Second approach: 35 SNPs were selected based on the overall genetic effect in exposed subjects ($p < 10^{-4}$). The most pronounced genetic effect was observed for rs5011804 on chromosome 12 (discovery meta-analysis $OR_{int} = 1.50$, $p = 1.21 \times 10^{-4}$; replication: $OR_{int} = 1.40$, $p = 0.03$).

Conclusions

Using two genome-wide interaction approaches, we identified novel polymorphisms in non-annotated intergenic regions on chromosomes 9 and 12, that showed suggestive evidence for interaction with active tobacco smoking in the onset of adult asthma.

Introduction

Exposure to environmental tobacco smoke increases the risk to develop asthma in childhood [1]. However, the role of active tobacco smoking in the onset of adult asthma remains inconclusive. Current and former smokers have a lower lung function [2–4] and increased bronchial hyperresponsiveness [5], whereas active smoking increases asthma severity [6]. The evidence for new onset asthma after active tobacco smoking is less clear. Active tobacco smoking has been associated with the onset of adult asthma [7,8], but not in all studies [6,9,10]. It has been hypothesized that tobacco smoking moderates the immune system by increasing IgE levels, thereby contributing to asthma onset [11].

Asthma is a complex disease that is thought to be caused by an interaction of environmental exposures and genetic susceptibility. Active tobacco smoking may increase the risk for asthma in a susceptible population only. Two candidate gene studies have suggested an interaction between active tobacco smoking and genetic variants in the occurrence of asthma in adults, i.e. the genes thymic stromal lymphopoietin (*TSLP*) [12] and filaggrin (*FLG*) [13]. Similarly, a study showed an interaction between active tobacco smoking and genes involved in lung function decline [14]. Above studies were based on hypothesis driven gene selection. One genome-wide association study on adult onset asthma, with a hypothesis free design, revealed that polymorphisms in the *HLA-DQ* gene increase the risk for adult onset asthma [15], an effect that was independent of tobacco smoke exposure.

Insight in the interaction between active tobacco smoking and genetic susceptibility is crucial for further development on knowledge on the etiology of adult onset asthma and for the development of effective strategies for asthma prevention. We therefore performed a genome-wide interaction (GWI) analysis using data of studies participating in the GABRIEL consortium [15]. We replicated our top hits in a large population study in the Northern part of the

Netherlands: LifeLines Cohort Study [16]. We set out to identify new genetic variants that interact with active tobacco smoking with respect to asthma onset at adult age.

Methods

Subjects

Data from six individual studies selected on presence of adult onset asthma data were included in the discovery meta-analysis on the interaction between single nucleotide polymorphisms (SNPs) and ever active tobacco smoking (Fig 1, S1 and S2 Checklists). All cases and controls

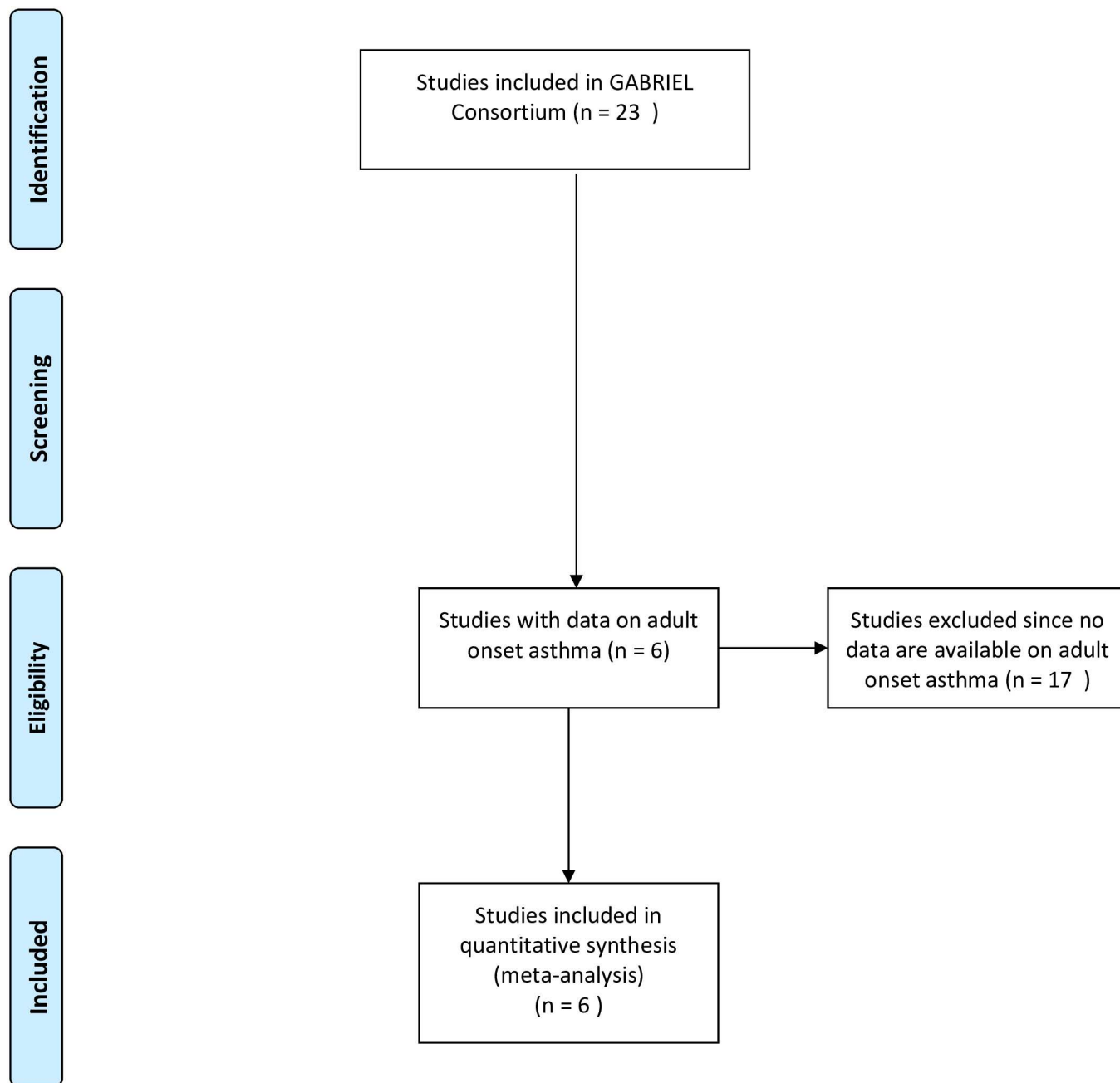


Fig 1. PRISMA flow diagram.

doi:10.1371/journal.pone.0172716.g001

were of European descent and two studies had a family structure. The study was approved by the local Medical Ethical Review Committees and all subjects gave written informed consent (Description of studies and ethical approval in the supporting information ([S1 File](#))). Adult onset asthma was defined as asthma diagnosed by a doctor when the subject was 16 years of age or older, as defined within the GABRIEL consortium [15]. Controls were all free of asthma, including childhood onset asthma. Active tobacco smoking was defined as 'ever active tobacco smoking'. Details on the outcome and exposure definition for the individual studies can be found in the [S1 File](#).

Genotyping and quality control

Genotyping was performed using the Illumina Human610 quad array (www.illumina.com) at CEA-Centre National de Génotypage, Evry, France. Details on the genotyping method have been described previously [15]. We restricted our meta-analyses to SNPs fulfilling the following quality control criteria in each study: genotype missing rate <3% in cases and controls, minor allele frequency >5% in controls and consistency with Hardy-Weinberg equilibrium in controls ($p\text{-value} > 10^{-4}$). Samples with >95% genotyping success rate were included in the analyses. We excluded putative non-European samples, identified using EIGENSTRAT2.0 software.

Statistical analyses

All individual studies were analysed using a logistic regression model with adult onset asthma as outcome. For each individual study a genome wide analysis on adult onset asthma was performed using logistic regression analysis including the SNP, ever active tobacco smoking, as well as the interaction between the SNP and ever active tobacco smoking to assess whether the effect of smoking on adult asthma differed between subjects with different genotypes. Also a stratified analysis was performed to analyse the genetic effect in exposed and non-exposed subjects. In all models an additive genetic model was used. Gender, age and informative principal components for within-Europe diversity were included as covariates. For the studies containing family data, a cluster variable indicating the family relations was included.

We meta-analysed the results of the individual studies (discovery meta-analysis) and used two selection procedures to identify SNPs that interact with ever active tobacco smoking in the adult onset asthma. To assess heterogeneity Cochran's Q statistic was calculated of each SNP and a random effect model was fitted.

Firstly, we followed the classical GWI study approach that is based on selection of the most significant interaction effect, i.e. the overall difference between the genetic effect in smokers and non-smokers with the lowest p-value. With this approach, smaller genetic effects occurring only after exposure to active tobacco smoking can be missed. For that reason we also followed a second approach where we selected genetic markers that are significantly associated with adult onset asthma in exposed subjects, but not in non-exposed subjects.

In the first approach we meta-analysed the study specific interaction effects and we selected SNPs with a fixed effect meta-analysis interaction effect with $p\text{-value} < 10^{-4}$. In the second approach we meta-analysed the genetic main effect in exposed and non-exposed subjects separately and we then selected SNPs with a genetic effect with $p\text{-value} < 10^{-4}$ only in exposed subjects based on the fixed effect model. SNPs with the same effect in exposed and non-exposed subjects were omitted by filtering on a nominal interaction effect ($p\text{-value} > 10^{-2}$).

Only SNPs present in at least two studies were included in the discovery meta-analysis, yielding to a total of 525,150 SNPs. Genome wide significance was set to a $p\text{-value} < 9.5 \times 10^{-8}$ based on Bonferroni correction. All SNPs selected from the discovery meta-analysis were

tested for replication in an independent population, the LifeLines Cohort Study [16] (Description of study in S1 File).

To investigate if the association between genetic background, tobacco smoking and adult onset asthma was robust for the different smoking habits we assessed the genetic effects of the identified SNPs on adult onset asthma in different strata of smoking habits (ever, current and former active smoking, as well as current passive smoking) in the LifeLines cohort study: exposed versus non-exposed to ever active tobacco smoking; exposed versus non-exposed to current active tobacco smoking; exposed versus non-exposed to active smoking in the past; exposed versus non-exposed to current passive smoking (details on the exposure definitions in S1 File). The analyses were conducted using Plink 1.07 [17] and R [18]. For annotation and inspection of linkage disequilibrium (LD) patterns WGAviewer [19] was used.

Results

The discovery genome-wide interaction meta-analysis consisted of 1,324 cases and 2,733 controls derived from six studies (Table 1). Overall, active tobacco smoking was not associated with adult onset asthma (Fig 2).

Firstly, we identified 50 SNPs in the discovery meta-analysis with an interaction p -value $< 10^{-4}$. None of the SNPs reached genome-wide significance. The results for two SNPs showed heterogeneity across studies (p -value Q -statistic < 0.05); these SNPs were omitted from further analysis. In the replication study, 29 of the 48 SNPs were included since 19 SNPs were not successfully imputed in the LifeLines Cohort Study or did not pass quality control (S1 Table). In total, 16 SNPs showed the same direction of the interaction effect in the discovery and replication analysis. None of the associations reached statistical significance in the replication study after Bonferroni correction for multiple testing for 29 SNPs (p -value < 0.0017) (Table 2). One SNP reached nominal significance: rs9969775 on chromosome 9. For this SNP the interaction estimate in the discovery meta-analysis was $OR_{int} = 0.50$, p -value = 7.63×10^{-5} and in the replication study: $OR_{int} = 0.65$, p -value = 0.02 (Table 2). Fig 3 shows the forest plots with the results for the discovery studies. In the smoking stratified analysis, non-exposed subjects carrying an A allele tended to have an increased asthma risk (discovery meta-analysis $OR = 1.57$, p -value = 1.88×10^{-3} , replication study $OR = 1.20$, p -value = 0.19), which was not observed in exposed subjects.

Table 1. Study populations included in GWI study on active smoking and adult onset asthma.

Study	Country	Design	Ever tobacco smokers, % (N)	N	Cases			Controls		
					Total	Exposed	(%)	Total	Exposed	(%)
Discovery study										
B58C	UK	Cohort	27.2 (123)	452	232	63	27.2	220	60	27.3
ECRHS	European	Multicentre	57.4 (710)	1238	353	196	55.5	885	514	58.1
EGEA	France	Cohort, family structure	49.5 (407)	822	186	90	48.4	636	317	49.8
KSMU	Russia	Case-control	64.2 (255)	397	164	110	67.1	233	145	62.2
SAPALDIA	Switzerland	Cohort	55.9 (498)	891	354	201	56.8	537	297	55.3
TOMSK	Russia	Cohort, family structure	44.4 (114)	257	35	13	37.1	222	101	45.5
TOTAL			51.9 (2107)	4057	1324	673	48.7	2733	1434	49.7
Replication study										
LifeLines	Netherlands	Cohort	60.1% (7496)	12475	366	225	61.5	12109	7271	60.0

Numbers are shown for subjects who were successfully genotyped and whose genotypes passed all quality checks

doi:10.1371/journal.pone.0172716.t001

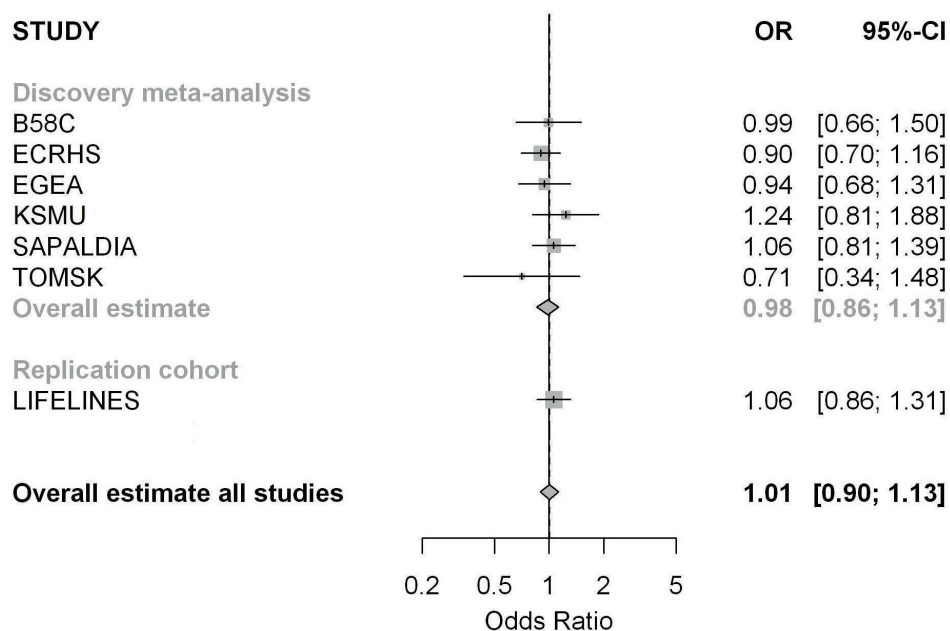


Fig 2. Forest plot for meta-analysis on the association between ever active tobacco smoking and adult onset asthma, without including the genetic effect.

doi:10.1371/journal.pone.0172716.g002

Secondly, we identified 35 SNPs in the discovery meta-analysis with a genetic effect of $p\text{-value} < 10^{-4}$ and an interaction $p\text{-value} < 10^{-2}$. Findings did not reach genome-wide significance. None of the SNPs showed heterogeneity across studies ($p\text{-value}$ Q-statistic < 0.05). In the replication study, 27 of the 35 SNPs were included, since 8 SNPs were not successfully imputed in the LifeLines Cohort Study or did not pass quality control (S1 Table). For 15 SNPs, the direction of the effect in the exposed subjects was the same in the discovery and replication analysis. None of the associations reached statistical significance in the replication study after Bonferroni correction for multiple testing for 27 SNPs ($p\text{-value} < 0.0019$) (Table 3). One SNP reached nominal significance in the replication: rs5011804 on chromosome 12 ($OR_{\text{int}} = 1.40$, $p\text{-value} = 0.03$). The interaction estimate for this SNP was $OR_{\text{int}} = 1.50$, $p\text{-value} = 1.21 \times 10^{-4}$ in the discovery meta-analysis (Table 3). Fig 4 shows the forest plots with results for the individual studies. In subjects who ever smoked, carriers of the minor allele C had an increased risk for asthma (discovery meta-analysis $OR = 1.42$, $p\text{-value} = 1.56 \times 10^{-6}$; replication study $OR = 1.21$, $p\text{-value} = 0.05$), while in non-exposed subjects, carriers of the C allele had no increased asthma risk (discovery meta-analysis $OR = 0.92$, $p\text{-value} = 0.31$, replication study $OR = 0.86$, $p\text{-value} = 0.24$).

Four SNPs were identified by both approaches (Table 4), but the results for these SNPs could not be replicated in LifeLines Cohort Study. The S2 Table shows the annotation of all SNPs identified in at least one of the approaches.

The analyses of the robustness of the results showed that the identified SNPs interacted with active tobacco smoking and not with passive smoking (Table 5), effects being particularly apparent among ex-smokers.

Discussion

This study is the first hypothesis-free genome-wide study specifically aiming to identify SNPs that interact with active tobacco smoking with respect to asthma onset at adult age. The results

Table 2. Top SNPs that interact with active tobacco smoking in adult onset asthma identified in first approach (overall interaction effect)[#].

Ch	SNP	Position	Effect allele	MAF*	Discovery meta-analysis				Replication study								Direction of the effect [¶]	
					Interaction		Exposed		Interaction		Exposed							
					OR _{int} [§]	95%CI	P [§]	OR [§]	95%CI	P [§]	OR _{int} [§]	95%CI	P [§]	OR [§]	95%CI	P [§]		
	rs4926457	245012644	C	0.42	0.65	0.52;0.80	4.49E-05	0.81	0.70;0.94	4.42E-03	1.03	0.76;1.39	0.86	1.06	0.88;1.28	0.54	-/+	
	rs10924824	244998447	G	0.42	0.64	0.52;0.79	2.96E-05	0.81	0.70;0.93	3.79E-03	0.99	0.74;1.34	0.97	1.05	0.87;1.27	0.61	-/+	
	rs4244627	245007487	G	0.42	0.64	0.52;0.79	3.52E-05	0.81	0.70;0.93	3.99E-03	1.02	0.75;1.38	0.91	1.05	0.87;1.27	0.62	-/+	
	rs10924823	244998415	T	0.42	0.65	0.52;0.80	4.28E-05	0.81	0.70;0.94	5.22E-03	0.99	0.73;1.34	0.96	1.05	0.87;1.27	0.62	-/+	
	rs1448187	111936830	T	0.28	0.65	0.53;0.81	9.34E-05	0.83	0.71;0.97	1.84E-02	1.04	0.75;1.45	0.81	1.06	0.87;1.31	0.55	-/+	
	rs2195614	221958757	A	0.43	1.51	1.23;1.85	8.10E-05	1.13	0.98;1.30	8.37E-02	1.04	0.77;1.41	0.80	1.04	0.86;1.25	0.70	+/+	
	rs2217431	221967489	A	0.43	1.53	1.24;1.88	5.14E-05	1.14	0.99;1.32	6.91E-02	1.04	0.77;1.41	0.80	1.04	0.86;1.25	0.70	+/+	
	rs13000320	237388433	C	0.17	0.57	0.44;0.76	9.00E-05	0.82	0.67;0.99	4.28E-02	0.86	0.58;1.28	0.46	0.92	0.72;1.19	0.55	-/-	
	rs428834	1629347	T	0.08	2.32	1.55;3.48	4.06E-05	1.57	1.22;2.03	5.68E-04	1.37	0.79;2.38	0.26	1.29	0.93;1.77	0.12	+/+	
	rs6863550	174552023	A	0.35	0.63	0.50;0.78	2.55E-05	0.75	0.64;0.87	1.89E-04	1.03	0.75;1.41	0.87	1.13	0.93;1.38	0.23	-/+	
	rs943801	165912238	C	0.21	0.59	0.46;0.77	6.69E-05	0.75	0.63;0.90	2.20E-03	1.00	0.70;1.42	0.99	1.13	0.91;1.41	0.27	-0	
	rs2987296	165927063	T	0.14	0.52	0.38;0.71	3.35E-05	0.74	0.60;0.92	6.73E-03	1.14	0.76;1.70	0.53	1.12	0.87;1.43	0.37	-/+	
	rs643066	165872834	T	0.25	0.58	0.46;0.74	1.19E-05	0.79	0.67;0.94	6.77E-03	1.18	0.84;1.66	0.35	1.28	1.04;1.57	0.02	-/+	
	rs2988576	12352801	A	0.46	0.66	0.54;0.81	5.37E-05	0.75	0.65;0.87	1.13E-04	1.15	0.83;1.58	0.41	1.01	0.82;1.23	0.96	-/+	
	rs9969775	13561933	A	0.13	0.50	0.35;0.70	7.63E-05	0.84	0.65;1.07	1.50E-01	0.65	0.45;0.93	0.02	0.78	0.61;1.00	0.05	-/-	
	rs4338205	17736447	A	0.11	2.11	1.47;3.02	5.04E-05	1.46	1.16;1.84	1.18E-03	0.61	0.36;1.04	0.07	0.79	0.54;1.14	0.20	+/-	
	rs4745437	77497877	C	0.43	0.62	0.50;0.76	6.00E-06	0.82	0.71;0.95	7.69E-03	0.89	0.65;1.23	0.49	0.89	0.73;1.08	0.25	-/-	
	rs1328550	77499107	C	0.33	1.55	1.25;1.92	7.28E-05	1.21	1.05;1.41	1.04E-02	1.00	0.72;1.38	1.00	1.07	0.88;1.31	0.48	+/+	
	rs7074731	23142594	C	0.17	1.79	1.35;2.37	5.94E-05	1.32	1.10;1.59	2.93E-03	1.04	0.67;1.63	0.85	0.89	0.68;1.17	0.42	+/-	
	rs999481	5363096	G	0.42	0.66	0.53;0.81	8.26E-05	0.75	0.65;0.87	1.42E-04	0.75	0.55;1.03	0.07	0.85	0.70;1.04	0.11	-/-	
	rs1716466	118348243	G	0.41	1.52	1.24;1.87	6.66E-05	1.22	1.06;1.41	5.92E-03	0.96	0.70;1.30	0.77	1.08	0.89;1.31	0.44	+/+	
	rs7954580	128647904	A	0.14	0.55	0.41;0.74	9.99E-05	0.76	0.61;0.94	1.26E-02	0.94	0.61;1.46	0.79	0.90	0.69;1.19	0.46	-/-	
	rs9591994	58855563	C	0.33	0.64	0.52;0.80	8.09E-05	0.75	0.64;0.88	4.28E-04	0.76	0.39;1.49	0.42	1.01	0.64;1.59	0.96	-/+	
	rs9544173	75597382	G	0.08	0.40	0.27;0.59	5.67E-06	0.73	0.55;0.97	2.97E-02	0.74	0.31;1.75	0.50	0.96	0.54;1.73	0.90	-/-	
	rs8047401	79304514	T	0.35	0.66	0.53;0.81	9.98E-05	0.76	0.65;0.88	1.99E-04	0.88	0.62;1.25	0.47	0.87	0.71;1.08	0.22	-/-	
	rs11077501	66037656	C	0.36	0.65	0.53;0.80	6.60E-05	0.83	0.71;0.96	1.33E-02	0.83	0.61;1.13	0.24	1.04	0.86;1.27	0.68	-/+	
	rs1984399	40312545	A	0.42	1.59	1.29;1.96	1.31E-05	1.25	1.08;1.44	2.19E-03	0.68	0.50;0.93	0.02	0.89	0.73;1.09	0.26	+/-	
	rs727336	40318090	T	0.42	1.55	1.26;1.91	3.58E-05	1.24	1.07;1.43	3.56E-03	0.65	0.48;0.88	0.01	0.88	0.72;1.07	0.21	+/-	
	rs4553919	24941463	T	0.18	0.57	0.44;0.75	4.40E-05	0.80	0.66;0.98	2.71E-02	0.95	0.66;1.37	0.79	1.04	0.83;1.31	0.73	-/+	

[#] Selection based on interaction effect with active tobacco smoking. Additive genetic model. Interaction model included genetic effect, smoking effect, interaction effect, gender, age and informative principal components. Ch: Chromosome; OR: Odds ratio; OR_{int}: Interaction Odds ratio; CI: Confidence interval; P: p-value

* MAF: Minor allele frequency (%), median of MAF in all discovery studies;

§ OR and p-value are based on fixed effect model

¶ Direction of the effect: + = positive, — = negative, 0 = no association, D/R: Discovery meta-analysis/Replication study

doi:10.1371/journal.pone.0172716.t002

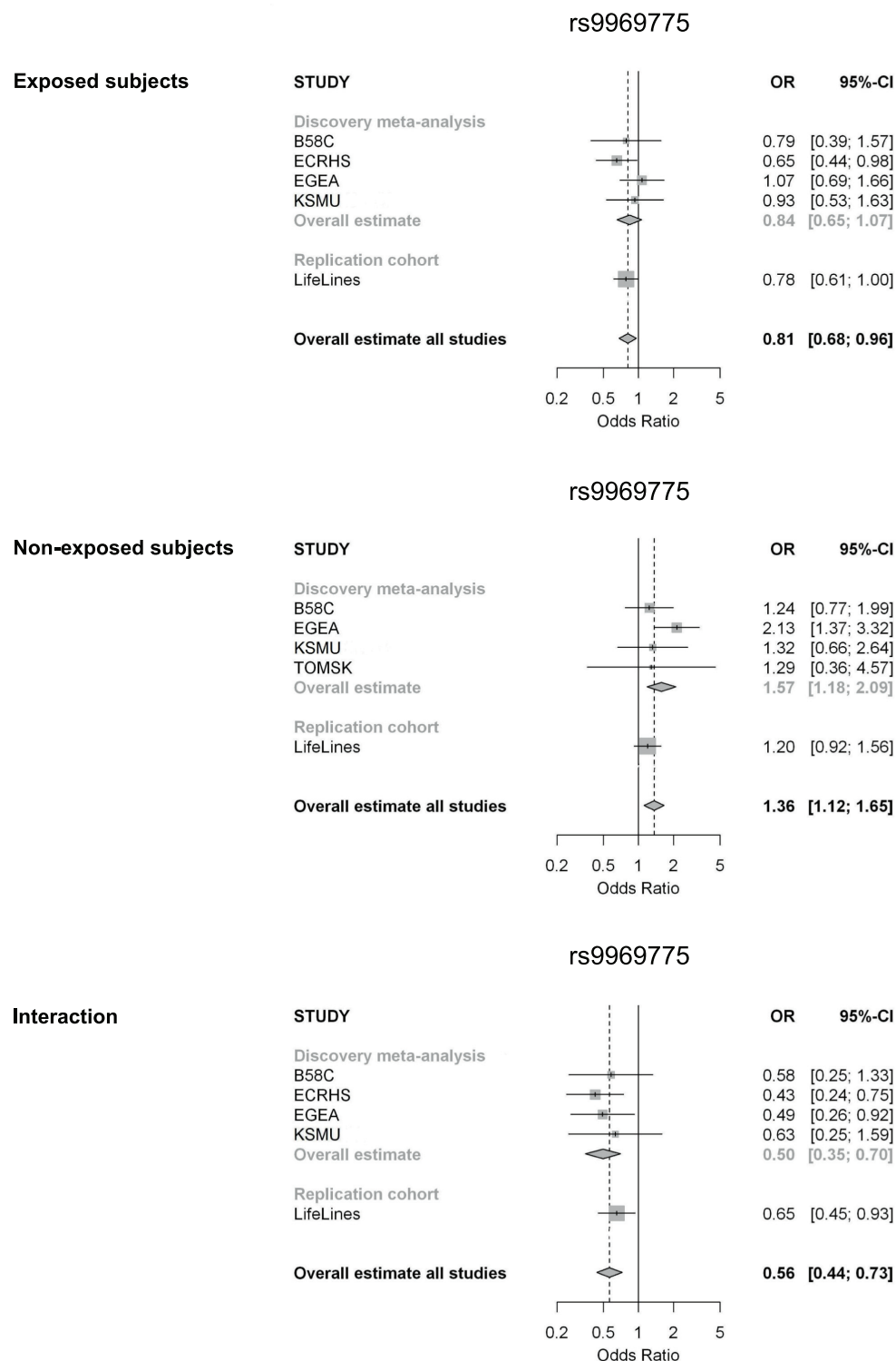


Fig 3. Forest plots for the meta-analysis and replication study on the genetic effect of SNP rs9969775 on chromosome 9 in subjects exposed and non-exposed to ever active tobacco smoking (identified in first approach). The bottom forest plot presents the interaction meta-analysis and replication study for this SNP. ORs are calculated using a fixed effect model.

doi:10.1371/journal.pone.0172716.g003

Table 3. Top SNPs that interact with active tobacco smoking in adult onset asthma identified in second approach (genetic effect in exposed)*.

Ch	SNP	Position	Effect allele	MAF*	Discovery meta-analysis				Replication study								Direction of the effect [†]	
					Interaction				Exposed				Interaction				Int	Exp
					OR _{int} [§]	95%CI	P [§]	OR [§]	95%CI	P [§]	OR _{int} [§]	95%CI	P [§]	OR [§]	95%CI	P [§]		
1	rs7513225	20634784	A	0.38	0.72	0.58;0.89	1.88E-03	0.74	0.64;0.86	7.88E-05	0.98	0.68;1.42	0.93	1.15	0.92;1.45	0.23	-/-	-/+
3	rs9758775	163957528	C	0.27	0.52	0.34;0.79	2.14E-03	0.48	0.34;0.67	2.31E-05	1.45	1.01;2.08	0.04	1.06	0.86;1.31	0.57	-/+	-/+
5	rs3853475	141796799	C	0.38	1.43	1.16;1.76	7.88E-04	1.36	1.18;1.57	3.68E-05	1.26	0.93;1.72	0.14	1.06	0.88;1.28	0.56	+/+	+/+
6	rs1106841	43604640	C	0.38	1.44	1.16;1.77	7.36E-04	1.35	1.17;1.56	4.17E-05	0.94	0.69;1.28	0.70	1.05	0.87;1.28	0.58	+/-	+/+
6	rs2812719	80410202	A	0.41	1.38	1.12;1.69	2.03E-03	1.38	1.20;1.59	9.39E-06	1.08	0.79;1.47	0.63	1.12	0.93;1.36	0.23	+/+	+/+
6	rs723981	80421994	T	0.07	1.80	1.24;2.63	2.22E-03	1.77	1.37;2.27	9.74E-06	0.52	0.33;0.85	0.01	0.83	0.59;1.17	0.28	+/-	+/-
6	rs1883877	80439582	A	0.07	1.86	1.27;2.71	1.36E-03	1.77	1.37;2.27	9.74E-06	0.82	0.43;1.57	0.55	0.80	0.53;1.21	0.28	+/-	+/-
7	rs2015523	88616537	T	0.16	1.51	1.12;2.02	6.11E-03	1.52	1.23;1.86	7.31E-05	1.06	0.69;1.63	0.78	0.96	0.74;1.25	0.77	+/+	+/-
8	rs7816370	3037931	A	0.17	0.68	0.51;0.90	6.18E-03	0.66	0.54;0.79	1.51E-05	1.02	0.68;1.52	0.93	0.96	0.75;1.23	0.74	-/+	-/-
8	rs17601573	87135695	C	0.54	1.37	1.11;1.68	2.67E-03	1.36	1.18;1.56	2.04E-05	1.13	0.84;1.53	0.42	1.11	0.92;1.34	0.27	+/+	+/+
9	rs2890993	14741872	G	0.15	1.66	1.23;2.25	8.94E-04	1.52	1.25;1.85	3.76E-05	1.27	0.79;2.02	0.32	1.12	0.85;1.47	0.42	+/+	+/+
9	rs17061224	77273982	T	0.13	1.47	1.10;1.97	9.79E-03	1.49	1.23;1.82	6.47E-05	1.27	0.76;2.13	0.36	1.12	0.83;1.52	0.46	+/+	+/+
10	rs7906433	3878845	T	0.24	0.64	0.50;0.83	4.30E-04	0.70	0.60;0.83	3.91E-05	1.21	0.86;1.71	0.28	1.11	0.90;1.37	0.33	-/+	-/+
11	rs3818275	35265359	C	0.34	0.66	0.53;0.82	1.87E-04	0.74	0.63;0.86	7.11E-05	1.05	0.76;1.45	0.76	1.08	0.89;1.32	0.44	-/+	-/+
12	rs11047993	25439546	A	0.46	1.45	1.18;1.78	3.86E-04	1.41	1.22;1.63	2.52E-06	1.31	0.97;1.78	0.08	1.17	0.97;1.41	0.10	+/+	+/+
12	rs11047994	25439598	A	0.40	1.43	1.17;1.77	6.70E-04	1.35	1.16;1.55	5.34E-05	1.31	0.96;1.79	0.09	1.17	0.97;1.41	0.10	+/+	+/+
12	rs4578491	25440513	A	0.46	1.47	1.19;1.80	2.64E-04	1.43	1.24;1.65	1.34E-06	1.31	0.97;1.78	0.08	1.17	0.97;1.41	0.10	+/+	+/+
12	rs5011804	25441894	C	0.46	1.50	1.22;1.84	1.21E-04	1.42	1.23;1.65	1.56E-06	1.40	1.03;1.90	0.03	1.21	1.00;1.46	0.05	+/+	+/+
13	rs4884334	58839005	G	0.32	0.70	0.56;0.87	1.78E-03	0.72	0.61;0.85	9.66E-05	1.35	0.95;1.92	0.09	1.05	0.85;1.29	0.67	-/+	-/+
17	rs8071270	66907543	T	0.29	0.67	0.53;0.84	5.28E-04	0.73	0.62;0.85	8.68E-05	0.97	0.68;1.39	0.87	0.97	0.77;1.21	0.79	-/-	-/-
17	rs7226071	66917957	G	0.29	0.67	0.54;0.85	6.61E-04	0.73	0.62;0.85	9.76E-05	0.89	0.62;1.29	0.56	0.93	0.74;1.17	0.53	-/-	-/-
17	rs6501483	66920291	G	0.29	0.67	0.54;0.85	6.74E-04	0.73	0.62;0.85	8.58E-05	0.99	0.71;1.40	0.98	0.99	0.80;1.23	0.93	-/-	-/-
17	rs2367536	66975870	C	0.29	0.72	0.57;0.90	4.76E-03	0.72	0.62;0.85	6.83E-05	1.05	0.73;1.52	0.78	0.95	0.76;1.19	0.66	-/+	-/+
18	rs724676	5916216	T	0.53	0.70	0.58;0.86	6.31E-04	0.76	0.66;0.87	9.28E-05	0.95	0.70;1.28	0.73	1.00	0.83;1.21	0.97	-/-	-/0
19	rs618940	39328412	G	0.39	0.71	0.57;0.87	9.95E-04	0.74	0.64;0.85	2.93E-05	1.33	0.97;1.83	0.07	1.23	1.02;1.49	0.03	-/+	-/+
20	rs6072658	40278039	C	0.19	0.61	0.47;0.78	1.51E-04	0.69	0.58;0.83	9.38E-05	1.61	1.10;2.35	0.01	1.20	0.96;1.49	0.11	-/+	-/+
20	rs10485689	40312475	T	0.19	0.60	0.46;0.78	1.28E-04	0.68	0.56;0.82	4.23E-05	1.40	0.98;2.00	0.07	1.17	0.95;1.46	0.15	-/+	-/+

Selection based on genetic effect in subjects exposed to active tobacco smoking. Additive genetic model. Interaction model included genetic effect, smoking effect, interaction effect, gender, age and informative principal components. Ch: Chromosome; Ref allele: Reference allele; OR: Odds ratio; OR_{int}: Interaction Odds ratio; CI: Confidence interval; P: p-value

* MAF: Minor allele frequency (%), median of MAF in all discovery studies;

§ OR and p-value are based on fixed effect model

† Direction of the effect: + = positive, - = negative, 0 = no association, D/R: Discovery meta-analysis/Replication study

doi:10.1371/journal.pone.0172716.t003

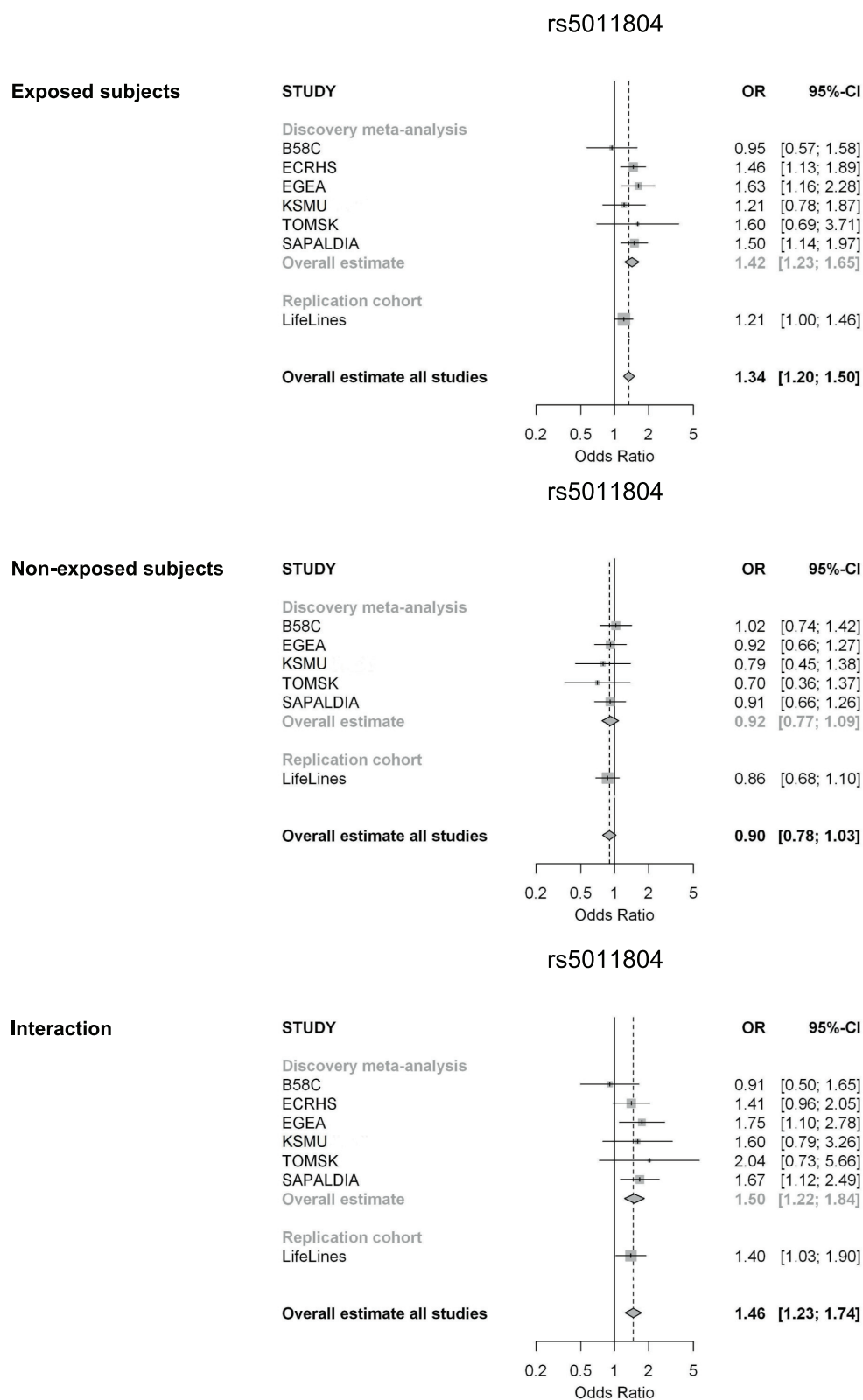


Fig 4. Forest plots for the meta-analysis and replication study on the genetic effect of SNP rs5011804 on chromosome 12 in subjects exposed and non-exposed to ever active tobacco smoking (identified in second approach). The bottom forest plot presents the interaction meta-analysis and replication study for this SNP. ORs are calculated using a fixed effect model.

doi:10.1371/journal.pone.0172716.g004

Table 4. Top SNPs that interact with active tobacco smoking in adult onset asthma identified in both approaches[#].

Ch	SNP	Position	Effect allele	MAF*	Discovery meta-analysis						Replication study						Direction of the effect [¶]
					Interaction			Exposed			Interaction			Exposed			
					OR _{Int} [§]	95%CI	P [§]	OR [§]	95%CI	P [§]	OR _{Int} [§]	95%CI	P [§]	OR [§]	95%CI	P [§]	
5	rs4912832	141632275	A	0.46	1.59	1.30;1.96	8.04E-06	1.38	1.20;1.59	1.01E-05	1.25	0.91;1.72	0.17	1.01	0.83;1.24	0.90	+/+
5	rs4541689	141631376	G	0.46	1.61	1.31;1.98	5.40E-06	1.39	1.20;1.60	7.39E-06	1.24	0.88;1.73	0.22	0.94	0.76;1.16	0.56	+/-
19	rs1759092	39368378	G	0.40	0.65	0.53;0.80	4.99E-05	0.75	0.65;0.87	9.79E-05	1.25	0.90;1.73	0.18	1.21	0.99;1.48	0.06	-/+
20	rs7262414	40245194	A	0.19	0.59	0.46;0.77	8.63E-05	0.68	0.57;0.82	6.33E-05	1.55	1.07;2.24	0.02	1.21	0.98;1.51	0.08	-/+

[#] Additive genetic model. Interaction model included genetic effect, smoking effect, interaction effect, gender, age and informative principal components.

Ch: Chromosome; Ref allele: Reference allele; OR: Odds ratio; OR_{int}: Interaction Odds ratio; CI: Confidence interval; P: p-value

* MAF: Minor allele frequency (%), median of MAF in all discovery studies;

§ OR and p-value are based on fixed effect model

¶ Direction of the effect: + = positive, - = negative, 0 = no association, D/R: Discovery meta-analysis/Replication study

doi:10.1371/journal.pone.0172716.t004

Table 5. Genetic effect of SNP rs5011804 following an additive model in the LifeLines cohort (N = 12,475), stratified by different tobacco smoke exposures.

Exposure	Stratum	N*	%	Genetic effect		
				OR	95% CI	p-value
Ever active tobacco smoking	Exposed	7496	60.1	1.21	1.00; 1.46	0.05
	Non-exposed	4979	39.9	0.86	0.68; 1.10	0.24
Current active tobacco smoking	Exposed	2800	22.5	0.84	0.61; 1.17	0.31
	Non-exposed	9666	77.5	1.14	0.97; 1.35	0.12
Ex smoker	Exposed	4624	37.1	1.44	1.14; 1.82	0.003
	Non-exposed	7842	62.9	0.89	0.73; 1.07	0.21
Current passive smoking	Exposed	2487	36.4	0.92	0.66; 1.27	0.61
	Non-exposed	4343	63.6	0.99	0.77; 1.28	0.96

* Numbers may not add up to 12,475, due to missing data on the specific exposure.

doi:10.1371/journal.pone.0172716.t005

are based on data from GABRIEL, a large consortium on adult onset asthma. We found suggestive evidence for an interaction between active tobacco smoking and rs9969775 on chromosome 9 and rs5011804 on chromosome 12. Both SNPs are intergenic markers that do not annotate to genes nor do SNPs in LD with these markers.

The SNPs found have not been identified previously in general GWA studies on asthma. Although the identified markers do not annotate for a protein coding region, they may have a regulatory function. rs9969775 is a tri-allelic polymorphism but in our datasets only two alleles were present (effect allele: A, reference allele: C). Rs9969775 is located between the *FLJ41200* gene (distance ~ 129 KB, also known as LINC01235) and *RP11-284P20.1* (distance ~ 366 KB). Both *FLJ41200* and *RP11-284P20.1* are long intergenic non-protein coding RNA genes. With the development of whole genome and transcriptome sequencing technologies, long noncoding RNAs have received increased attention. Multiple studies indicate that they can regulate gene expression in many ways, including chromatin modification, transcription and post-transcriptional processing [20]. A search for rs9969775 in the ENCODE database (using the WashU Epi Genome Browser <http://epigenomegateway.wustl.edu/>) showed that this SNP is located at a CpG site with a high methylation score in lung tissue. Further analysis of this SNP using Haploreg indicated that this SNP is located in a region of active chromatin in the lung, as indicated by a DNASE I hypersensitivity site, in an enhancer region (Haploreg version 4.1: <http://archive.broadinstitute.org/mammals/haploreg/haploreg.php>).

The second identified SNP, rs5011804, is located between the *KRAS* gene (distance ~ 38 KB) and the *RPL39P27* gene (distance ~ 120 KB). The *KRAS* gene encodes a protein that is a member of the small GTPase superfamily. Small GTPases regulate a wide variety of processes in the cell, including growth, cellular differentiation, cell movement and lipid vesicle transport. *RPL39P27* is a ribosomal protein pseudogene. Pseudogenes are fragments of genes that were functional but have been silenced by one or more mutations[21]. It was assumed that pseudogenes were not functional but recent studies suggest that they may have a functional role such as gene expression, gene regulation, and generation of genetic diversity [22]. Finally, to gain more insight in the possible regulatory roles of rs9969775 and rs5011804 on gene expression, data from the Genotype-Tissue Expression project (<http://www.gtexportal.org/home/>) was used. The results showed that the SNPs were not associated with gene expression of any gene in any tissue. In summary, our identified SNPs are located in regions with potential regulatory function and future research is needed to unravel their role in adult asthma further. Of interest, the two SNPs that were previously reported to be associated with adult onset asthma [15]

(rs17843604 and rs9273349 on chromosome 6) showed nominal significant associations with asthma in both smokers and non-smokers but no interaction with active tobacco smoking in our meta-analysis (S3 Table).

The GWI study design is specifically suited to identify novel SNPs that interact with an environmental exposure in an unbiased way. Genes identified to interact with active tobacco smoking are crucial for further insight in the etiology of adult onset asthma and development of effective strategies for asthma prevention. A strength of our study is that we followed two different approaches to detect SNPs that show a differential effect in subjects exposed and non-exposed to smoking. The classical GWI study approach is to select SNPs with the largest interaction effect. Since we also aimed to identify subpopulations that are genetically susceptible for active tobacco smoking we followed a second approach in which we selected SNPs that only affected the risk of asthma in exposed subjects and not in non-exposed subjects. In our analyses, four SNPs were identified with both approaches.

Since adult onset asthma is not common, only a subset of asthmatics is exposed, and the expected effect size is small, a large sample size is needed to obtain a genome-wide significant finding. In this study we combined data from multiple studies to achieve this. We additionally harmonized the exposure and outcome definitions in the different studies as much as possible to improve the chance of finding significant interactive effects. However, small differences in these definitions between studies could create random error which compromises study power and thus makes it harder to detect a significant interaction [23].

A limitation of our study is that active tobacco smoking is related to exposure to environmental smoke at different periods in life, which makes it difficult to disentangle the effects of these exposures. Therefore, we assessed the genetic effects of the identified SNPs on adult onset asthma in different strata of smoking habits in the LifeLines Cohort Study. Results showed that genetic effects of the identified SNPs were particularly apparent among ex smokers.

Two studies included in the meta-analysis contained cross-sectional and retrospectively collected data. In these studies, asthma onset before the start of smoking could not be ruled out. Inclusion of these subjects would lead to a dilution of the actual interaction between genetics and ever smoking on adult onset asthma. Since data from the LifeLines Cohort Study showed that only eight (3.6%) subjects out of 225 ever smoking adult onset asthmatics started smoking after the start of adult onset asthma (data not shown), it is unlikely that this issue biased our results.

A general problem in GWI studies is their limited power, due to often a small number of subjects with overlapping exposures and genotypes [24,25]. The power to detect an interaction can be increased by assessing the association between exposure and genotype in a case-only design or a two-step design [24,25]. A case-only design assumes that exposure and genotype are independent. We chose not to use this design given the known strong genetic component of smoking addiction, and relatively modest violations of this assumption can have a substantial impact on bias relating to the interaction parameters [26], hence leading to false positive or false negative findings [27]. In a two-step design the interaction is tested among a selection of SNPs. The method we used to detect interactions between exposure and genotype did not assume exposure and genotype independence nor did we a priori select SNPs. To limit the possibility to miss possible interaction effects, we first selected the most promising SNPs using an arbitrary threshold for interaction ($p < 10^{-4}$) and included them in a replication study. A similar approach has been used successfully in a GWI study on interaction between genetic markers and waist hip ratio on total serum cholesterol [28].

In summary, we performed two approaches for GWI analyses and identified SNPs on chromosome 9 and 12, both intergenic variants with potential regulatory functions. These are

novel SNPs, previously unidentified by regular genome-wide association and candidate gene studies that showed suggestive evidence for interaction with active tobacco smoking in adult onset asthma. We propose that future studies replicate our findings.

Supporting information

S1 File. Description of individual studies.

(DOC)

S1 Table. Complete results of all identified SNPs.

(XLS)

S2 Table. Annotation of the top SNPs identified in both approaches.

(DOC)

S3 Table. Results for rs17843604 and rs9273349.

(XLS)

S1 Checklist. PRISMA 2009 checklist.

(DOC)

S2 Checklist. Meta-analysis on genetic association studies checklist | PLOS ONE.

(DOCX)

Author Contributions

Conceptualization: JMV SS DSP GHK HMB.

Data curation: JMV SS DSP MFM DJ AR MW ERO EB FD RN VS AVP MS VPI IC MI AK NP-H LMO VPP EYB MBF IMN AMF WOCMC DPS GHK HMB.

Formal analysis: SS.

Funding acquisition: DSP GHK HMB MFM WOCMC.

Investigation: JMV SS DSP MFM DJ AR MW ERO EB FD RN VS AVP MS VPI IC MI AK NP-H LMO VPP EYB MBF IMN AMF WOCMC DPS GHK HMB.

Methodology: JMV SS DSP GHK HMB.

Project administration: MFM WOCMC SS.

Supervision: DSP GHK HMB JMV.

Visualization: SS JMV.

Writing – original draft: JMV SS DSP GHK HMB.

Writing – review & editing: JMV SS DSP MFM DJ AR MW ERO EB FD RN VS AVP MS VPI IC MI AK NP-H LMO VPP EYB MBF IMN AMF WOCMC DPS GHK HMB.

References

1. Scholtens S, Postma DS, Moffatt MF, Panasevich S, Granell R, Henderson AJ, et al. Novel childhood asthma genes interact with in utero and early-life tobacco smoke exposure. *J Allergy Clin Immunol*. 2014; 133(3):885–888. doi: [10.1016/j.jaci.2013.08.049](https://doi.org/10.1016/j.jaci.2013.08.049) PMID: [24315450](https://pubmed.ncbi.nlm.nih.gov/24315450/)
2. Burchfiel CM, Marcus EB, Curb JD, Maclean CJ, Vollmer WM, Johnson LR, et al. Effects of smoking and smoking cessation on longitudinal decline in pulmonary function. *Am J Respir Crit Care Med*. 1995; 151(6):1778–1785. doi: [10.1164/ajrccm.151.6.7767520](https://doi.org/10.1164/ajrccm.151.6.7767520) PMID: [7767520](https://pubmed.ncbi.nlm.nih.gov/7767520/)

3. Peat JK, Woolcock AJ, Cullen K. Decline of lung function and development of chronic airflow limitation: A longitudinal study of non-smokers and smokers in busselton, western australia. *Thorax*. 1990; 45(1):32–37. PMID: [2321175](#)
4. Lange P, Parner J, Vestbo J, Schnohr P, Jensen G. A 15-year follow-up study of ventilatory function in adults with asthma. *N Engl J Med*. 1998; 339(17):1194–1200. doi: [10.1056/NEJM199810223391703](#) PMID: [9780339](#)
5. Curjuric I, Zemp E, Dratva J, Ackerman-Liebrich U, Bridevaux PO, Bettschart RW, et al. Determinants of change in airway reactivity over 11 years in the SAPALDIA population study. *Eur Respir J*. 2011; 37(3):492–500. doi: [10.1183/09031936.00188609](#) PMID: [20530037](#)
6. Siroux V, Pin I, Oryszczyn MP, Le Moual N, Kauffmann F. Relationships of active smoking to asthma and asthma severity in the EGEA study. epidemiological study on the genetics and environment of asthma. *Eur Respir J*. 2000; 15(3):470–477. PMID: [10759439](#)
7. Vogelberg C, Hirsch T, Radon K, Dressel H, Windstetter D, Weinmayr G, et al. Leisure time activity and new onset of wheezing during adolescence. *Eur Respir J*. 2007; 30(4):672–676. doi: [10.1183/09031936.00152906](#) PMID: [17596269](#)
8. Strachan DP, Butland BK, Anderson HR. Incidence and prognosis of asthma and wheezing illness from early childhood to age 33 in a national british cohort. *BMJ*. 1996; 312(7040):1195–1199. PMID: [8634562](#)
9. McWhorter WP, Polis MA, Kaslow RA. Occurrence, predictors, and consequences of adult asthma in NHANESI and follow-up survey. *Am Rev Respir Dis*. 1989; 139(3):721–724. doi: [10.1164/ajrccm/139.3.721](#) PMID: [2923372](#)
10. Vesterinen E, Kaprio J, Koskenvuo M. Prospective study of asthma in relation to smoking habits among 14,729 adults. *Thorax*. 1988; 43(7):534–539. PMID: [3269222](#)
11. Oryszczyn MP, Annesi-Maesano I, Charpin D, Paty E, Maccario J, Kauffmann F. Relationships of active and passive smoking to total IgE in adults of the epidemiological study of the genetics and environment of asthma, bronchial hyperresponsiveness, and atopy (EGEA). *Am J Respir Crit Care Med*. 2000; 161(4 Pt 1):1241–1246. doi: [10.1164/ajrccm.161.4.9905027](#) PMID: [10764318](#)
12. Liu M, Rogers L, Cheng Q, Shao Y, Fernandez-Beros ME, Hirschhorn JN, et al. Genetic variants of TSLP and asthma in an admixed urban population. *PLoS One*. 2011; 6(9):e25099. doi: [10.1371/journal.pone.0025099](#) PMID: [21966427](#)
13. Berg ND, Husemoen LL, Thuesen BH, Hersoug LG, Elberling J, Thyssen JP, et al. Interaction between filaggrin null mutations and tobacco smoking in relation to asthma. *J Allergy Clin Immunol*. 2012; 129(2):374–80, 380.e1–2. doi: [10.1016/j.jaci.2011.08.045](#) PMID: [22088612](#)
14. Curjuric I, Imboden M, Nadif R, Kumar A, Schindler C, Haun M, et al. Different genes interact with particulate matter and tobacco smoke exposure in affecting lung function decline in the general population. *PLoS One*. 2012; 7(7):e40175. doi: [10.1371/journal.pone.0040175](#) PMID: [22792237](#)
15. Moffatt MF, Gut IG, Demenais F, Strachan DP, Bouzignou E, Heath S, et al. A large-scale, consortium-based genomewide association study of asthma. *N Engl J Med*. 2010; 363(13):1211–1221. doi: [10.1056/NEJMoa0906312](#) PMID: [20860503](#)
16. Scholtens S, Smidt N, Swertz MA, Bakker SJ, Dotinga A, Vonk JM, et al. Cohort Profile: LifeLines, a three-generation cohort study and biobank. *Int J Epidemiol*. 2015; 44(4):1172–1180. doi: [10.1093/ije/dyu229](#) PMID: [25502107](#)
17. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, et al. PLINK: A tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet*. 2007; 81(3):559–575. doi: [10.1086/519795](#) PMID: [17701901](#)
18. R Development Core Team. R Foundation for Statistical Computing. R: A language and environment for statistical computing. <http://www.R-project.org>. 2011.
19. Ge D, Zhang K, Need AC, Martin O, Fellay J, Urban TJ, et al. WGAViewer: Software for genomic annotation of whole genome association studies. *Genome Res*. 2008; 18(4):640–643. doi: [10.1101/gr.071571.107](#) PMID: [18256235](#)
20. Shi X, Sun M, Liu H, Yao Y, Song Y. Long non-coding RNAs: a new frontier in the study of human diseases. *Cancer Lett*. 2013; 339(2):159–66. doi: [10.1016/j.canlet.2013.06.013](#) PMID: [23791884](#)
21. Goodhead I, Darby AC. Taking the pseudo out of pseudogenes. *Curr Opin Microbiol*. 2014; 23C:102–109.
22. Balakirev ES, Ayala FJ. Pseudogenes: are they "junk" or functional DNA? *Annu Rev Genet*. 2003; 37:123–51. doi: [10.1146/annurev.genet.37.040103.103949](#) PMID: [14616058](#)
23. Bookman EB, McAllister K, Gillanders E, Wanke K, Balshaw D, Rutter J, et al. Gene-environment interplay in common complex diseases: Forging an integrative model-recommendations from an NIH workshop. *Genet Epidemiol*. 2011; 35(4):217–225. doi: [10.1002/gepi.20571](#) PMID: [21308768](#)

24. Mukherjee B, Ahn J, Gruber SB, Chatterjee N. Testing gene-environment interaction in large-scale case-control association studies: Possible choices and comparisons. *Am J Epidemiol*. 2012; 175(3):177–190. doi: [10.1093/aje/kwr367](https://doi.org/10.1093/aje/kwr367) PMID: [22199027](https://pubmed.ncbi.nlm.nih.gov/22199027/)
25. Ege MJ, Strachan DP, Cookson WO, Moffatt MF, Gut I, Lathrop M, et al. Gene-environment interaction for childhood asthma and exposure to farming in central europe. *J Allergy Clin Immunol*. 2011; 127(1):138–44, 144.e1–4. doi: [10.1016/j.jaci.2010.09.041](https://doi.org/10.1016/j.jaci.2010.09.041) PMID: [21211648](https://pubmed.ncbi.nlm.nih.gov/21211648/)
26. Hunter DJ. Gene-environment interactions in human diseases. *Nat Rev Genet*. 2005; 6(4):287–298. doi: [10.1038/nrg1578](https://doi.org/10.1038/nrg1578) PMID: [15803198](https://pubmed.ncbi.nlm.nih.gov/15803198/)
27. Liu X, Fallin MD, Kao WH. Genetic dissection methods: Designs used for tests of gene-environment interaction. *Curr Opin Genet Dev*. 2004; 14(3):241–245. doi: [10.1016/j.gde.2004.04.011](https://doi.org/10.1016/j.gde.2004.04.011) PMID: [15172665](https://pubmed.ncbi.nlm.nih.gov/15172665/)
28. Surakka I, Isaacs A, Karssen LC, Laurila PP, Middelberg RP, Tikkanen E, et al. A genome-wide screen for interactions reveals a new locus on 4p15 modifying the effect of waist-to-hip ratio on total cholesterol. *PLoS Genet*. 2011; 7(10):e1002333. doi: [10.1371/journal.pgen.1002333](https://doi.org/10.1371/journal.pgen.1002333) PMID: [22028671](https://pubmed.ncbi.nlm.nih.gov/22028671/)

Supporting information

Adult onset asthma and interaction between genes and active tobacco smoking:

the GABRIEL consortium.

Vonk et al.

Description of individual studies

British 1958 birth cohort (B58C)

The 1958 British birth cohort is an ongoing follow-up of persons born in Britain during one week in 1958 (<http://www.b58cgene.sgul.ac.uk/>). At age 44-45 years, a full biomedical examination was performed from which DNA samples were prepared for use as a nationally representative reference series for genetic case-control studies.¹ About half of the cohort members with a history of asthma ascertained at any age up to 42 years, and a similar number of non-asthmatic controls, were included in the GABRIEL meta-analysis². For the purpose of this interaction analysis, adulthood asthmatics were defined as persons reporting asthma ever at any follow-up from 16 years of age. Controls were defined with exclusion of childhood onset case. Ever active smoking was ascertained by self-report and interview at age 23, 33 and 42 years.

ECRHS

Sixteen centres (eight countries) in the European Community Respiratory Health Survey (ECRHS) have contributed samples to GABRIEL (<http://www.ecrhs.org>).^{3,4} In each centre, a representative community-based sample of at least 3000 adults aged 20-44 years were invited to complete a brief postal questionnaire asking about respiratory symptoms (ECRHS I - Stage 1) between 1991-1993. A random sample of these (600 per centre) underwent intensive further investigation (ECRHS I - Stage 2 – random sample). Participants who had symptoms highly suggestive of asthma but who had not been selected at random to take part in Stage 2, were also invited to undergo intensive investigations (ECRHS I - Stage 2- enriched sample). About ten years later all adults who had taken part in Stage 2 were recontacted (ECRHS II) and again asked about respiratory symptoms. Samples suitable for DNA extraction were collected. For the GABRIEL initiative all cases of asthma were identified (participants

from the random or enriched sample who said yes to the question „Have you ever had asthma? at either ECRHS I or ECRHS II). Adulthood onset asthma cases were defined as asthma from 16 years of age. Active smoking was defined as “Ever active smoking by anyone from 16 years of age”.

EGEA

EGEA is a 12-year longitudinal survey which combines a case-control study and a family study (<https://egeanet.vjf.inserm.fr/>). The first survey (EGEA1) took place between 1992 and 1995.⁵ The study population included 388 asthmatics recruited in chest clinics and their 1,244 family members plus 415 population-based controls (total of 2,047 subjects). The probands (asthmatics and controls) were between 7 and 70 years old at time of study. All probands and their two parents were of European ancestry and were born in France. The second survey (EGEA2) was conducted between 2003 and 2007 and included follow-up data in 1,543 subjects from the initial cohort and 73 new family members.⁶ Data collected through face-to-face interviews and examination included extensive phenotypic characterization (detailed clinical data based on standardized questionnaire, skin prick tests, lung function tests, bronchial responsiveness, blood samples, white blood cell counts, total IgE), data on risk factors (environmental exposures, diet, physical activity, hormone-related events) and drug consumption. The study protocol was approved by the institutional ethics committee (baseline study: Cochin Port-Royal Hospital, Paris; follow-up: Necker-Enfants Malades Hospital, Paris), and all participants gave written informed consent. Asthma was defined with a positive answer to “Have you ever had attacks of breathlessness at rest with wheezing?” or “Have you ever had asthma attacks?” or being recruited as an asthma case in chest clinics. Adulthood asthma was defined as asthma onset from the age of 16 years. Controls were defined as subjects without asthma. Ever active smoking was defined as an affirmative answer on the question: "Do you smoke or have you ever smoked one cigarette per day or more for as long as a year? ".

Kursk State Medical University (KSMU)

KSMU is a population-based case-control study of adult cases of asthma and controls matched for age and sex.⁷ A total of 429 unrelated subjects were recruited in this study, (215 patients with asthma and 214 controls). The study subjects were of Russian origin from Central Russia. All patients were recruited at the Department of Pulmonology, Kursk Regional Clinical Hospital between 2003 and

2004. Additional adult patients with asthma and healthy subjects (>200 samples) from the same population were recruited between 2007 and 2008 specially in order to increase final sample size for the GWAS initiative. All patients were diagnosed with asthma by the presence of characteristic symptoms, reversibility of airway obstruction or airway hyperresponsiveness to methacholine. All control subjects were enrolled in accordance with the following criteria: (1) no symptoms and history of allergic diseases, (2) normal total serum IgE levels, (3) and normal pulmonary function test results. Childhood onset asthma cases were excluded from the analyses. Personal data, including smoking status and age of the disease onset, was collected through in-person interviews. Active smoking was defined as ever active smoking. The study was approved by the Ethical Review Committee of Kursk State Medical University, and the subjects who were recruited gave informed consent. After QC a total of 568 subjects were retained in the GWAS analyses.

SAPALDIA

SAPALDIA is a population-based cohort that originally recruited subjects aged 18 to 60 from population registries in eight Swiss communities.⁸ Subjects were obtained from among 6,055 SAPALDIA cohort subjects that participated in both, the baseline (1991) and follow-up (2002) examinations and agreed to providing blood for genetic analysis. At both baseline and follow-up examination subjects underwent spirometry as well as a detailed interview on respiratory health, smoking history and lifestyle factors. At follow-up examination, 8,047 of 9,651 baseline subjects re-participated in at least one part of the study and a formal biobank was established. SAPALDIA questions about smoking and asthma status were equivalent to those used by the ECRHS. Asthma status was defined by an affirmative answer to the question “Have you ever had asthma” at baseline and/or follow-up interview. Adulthood onset was defined as onset from 16 years of age. Controls were defined as subjects who never had asthma in their lifetime. A random sample was drawn from all controls with available GWAS data to match the proportional distribution of childhood and adulthood onset asthma. Ever active tobacco smoking was defined based on an affirmative answer to the question, whether the participant actively smoked or had been smoking in either of the surveys.

TOMSK

TOMSK is a population-based family study conducted by the Research Institute of Medical Genetics and Siberian State Medical University (Tomsk, Russia) from 1998 onwards.⁹ Both nuclear families and extended pedigrees were recruited through atopic bronchial asthmatic probands. All participants were Russians or of a mixed ethnic origin due to marriages between Russians and major East Slavonic populations (Ukrainians, Byelorussians). Altogether, 196 families were studied, out of which 150 families were recruited in Tomsk Region Children Hospital and Tomsk Region Hospital (Tomsk, Russia), and 46 families were recruited in the city of Irkutsk hospitals by the staff of the Irkutsk State Institute of Doctors Advanced Training (Irkutsk, Russia). Both probands and their relatives were clinically examined to establish diagnosis of asthma and atopy by the GINA criteria (Global Initiative for Asthma: Global Strategy for Asthma Management and Prevention. <http://www.ginasthma.org>). Besides the clinical examination, laboratory and functional testing were conducted to assess common IgE levels (solid-phase immune-enzyme assay), specific sensitization (skin-prick tests), lung volumes (spirometry), and airway responsiveness (bronchoprovocative tests with methacholine). Controls were defined with exclusion of childhood onset case. Active smoking was defined as ever active smoking.

LifeLines Cohort Study

The LifeLines Cohort Study is a three-generation cohort that is designed to investigate universal risk factors and their modifiers for multifactorial diseases.¹⁰ It is an observational follow-up study in a large representative sample of the population of the northern provinces of the Netherlands. Firstly, a random sample of persons aged between 25 and 50 years are contacted through their general practitioner and are invited to participate. Subsequently these probands invite their family members if present to take part as well (parents, partner, parents in law, children), resulting in a three-generation study. At enrollment subjects undergo a medical examination where blood sample is collected for DNA extraction. Participants filled in a questionnaire at baseline containing a question on whether they ever had asthma, whether the diagnosis has been confirmed by a physician and what was the age of onset. Adulthood onset asthma was defined as asthma onset from the age of 16 years. Ever active smoking was defined as any smoking at any age, starting before the onset of asthma. Current active tobacco smoking was defined as an affirmative answer on the question: "Do you smoke or have your smoked in the past month?". Current passive smoking was defined as "Were you exposed regularly in the

past 12 months to passive tobacco smoking?" Childhood onset cases were excluded from the analyses. Genotyping of 301,232 SNPs was performed with using Illumina HumanCytoSNP-12v2 array. Samples for 13,301 individuals were genotyped and passed QC. The data was imputed by BEAGLE 3.0.

Local Medical Ethical Review Committees

B58C	South East England Multi-Centre Research Ethics Committee and the National Research Ethics Service, London & South East Committee
ECRHS	NRES Committee London - Stanmore
EGEA	Institutional ethics committees of Cochin Port-Royal Hospital and Necker-Enfants Malades Hospital, Paris.
KSMU	Ethical Review Committee of Kursk State Medical University (KSMU)
SAPALDIA	Swiss Academy of Medical Sciences and ethics committees of all regional study sites (current appropriate cantonal ethics committees names are: Ethikkommission Nordwest- und Zentralschweiz, Commission cantonale d'éthique de la recherche de Genève, Kantonale Ethikkommission Zürich, Commission cantonale d'éthique de la recherche sur l'être humain, Comitato etico cantonale.)
TOMSK	Ethics Committees of the Research Institute for Medical Genetics and Siberian State Medical University, Tomsk (Russian Federation).
LifeLines	Medical ethical committee of the University Medical Center Groningen

Supplementary Methods

Genotyping and quality control

Genotyping of the GABRIEL study was performed using the Illumina Human610 quad array (www.illumina.com) at CEA-Centre National de Génotypage, Evry, France. Samples from cases and controls were randomly distributed on 96-well plates. Family relationships were confirmed or revised based on the results of an identity-by-state (IBS) analysis. An ancestry analysis was carried out using the EIGENSTRAT2.0 software and putative non-European samples were excluded from the analyses. The analyses were restricted to SNPs fulfilling the following quality control criteria: (1) genotype missing rate <3% in both cases and controls; (2) minor allele frequency $\geq$ 1% in controls; (3) consistency with Hardy-Weinberg equilibrium in controls ($P > 0.0001$). Informative principal components for within-Europe diversity were included as covariates in the association analysis.

Genotyping of the LifeLines study was performed with using Illumina HumanCytoSNP-12v2 array (www.illumina.com) at the Genotyping laboratory of the University Medical Center Groningen. Quality controls of the data are based on SNP filtering on minor allele frequency (MAF) above 0.01, Hardy-Weinberg equilibrium (HWE) P-value >0.0001 , call rate of 0.95 and principal component analysis (PCA) to check for population outliers. Only unrelated and Caucasian-ancestry samples were included in the analyses.

Acknowledgements

The GABRIEL study (a multidisciplinary study to identify the genetic and environmental causes of asthma in the European Community) was supported by the European Commission, contract number 018996 under the Integrated Program LSH-2004-1.2.5-1.

EGEA

We thank the EGEA cooperative group: **Coordination:** V Siroux (epidemiology, PI since 2013); F Demenais (genetics); I Pin (clinical aspects); R Nadif (biology); F Kauffmann (PI 1992-2012).

Respiratory epidemiology: Inserm U 700, Paris: M Korobaef (Egea1), F Neukirch (Egea1); Inserm U 707, Paris: I Annesi-Maesano (Egea1-2); Inserm CESP/U 1018, Villejuif: F Kauffmann, N Le Moual, R Nadif, MP Oryszczyn (Egea1-2), R Varraso; Inserm U 823, Grenoble: V Siroux. **Genetics:** Inserm U 393, Paris: J Feingold; Inserm U 946, Paris: E Bouzigon, F Demenais, MH Dizier; CNG, Evry: I Gut (now CNAG, Barcelona, Spain), M Lathrop (now Univ McGill, Montreal, Canada). **Clinical centers:** Grenoble: I Pin, C Pison; Lyon: D Ecochard (Egea1), F Gormand, Y Pacheco; Marseille: D Charpin (Egea1), D Vervloet (Egea1-2); Montpellier: J Bousquet; Paris Cochin: A Lockhart (Egea1), R Matran (now in Lille); Paris Necker: E Paty (Egea1-2), P Scheinmann (Egea1-2); Paris-Trousseau: A Grimfeld (Egea1-2), J Just. **Data and quality management:** Inserm ex-U155 (Egea1): J Hochez; Inserm CESP/U 1018, Villejuif: N Le Moual; Inserm ex-U780: C Ravault (Egea1-2); Inserm ex-U794: N Chateigner (Egea1-2); Grenoble: J Quentin-Ferran (Egea1-2). The authors thank all those who participated to the setting of the study and on the various aspects of the examinations involved: interviewers, technicians for lung function testing, coders, those involved in quality control, data management and all those who supervised the study in all centers. The authors are grateful to the three CIC-Inserm of Necker, Grenoble and Marseille who supported the study and in which subjects were examined. They are indebted to all the individuals who participated without whom that study would not have been possible.

EGEA sources of funding: INSERM-Ministry of Research 'Cohortes et Collections' grant (4CH06G). French Ministry of Higher Education and Research, University Paris Diderot-Paris 7, grants from the French Agency for Environmental and Occupational Health Safety (grant AFSSETAPR- SE-2004), the French National Agency for Research (grants ANR-05-SEST-020-02/05-9-97, ANR-06-CEBS and

ANR-11-BSV1-027-GWIS-AM), PHRC-Paris, Merck Sharp & Dohme (MSD)), the “Fonds de Dotation Recherche en Santé Respiratoire” (FRSR-AO-2011), Région Ile-de-France (DIM-SEnT 2011). For the genotyping (as for all Gabriel groups): The GABRIEL genotyping was supported by a contract from the European Commission Framework Programme 6 (018996) and grants from the French Ministry of Research.

Kursk State Medical University (KSMU)

The KSMU team is supported by the Federal Targeted Program “Scientific and Scientific-Pedagogical Personnel of the Innovative Russia“ of the Ministry of Education and Science of Russian Federation. The study was supported in part by a grant from the President of Russian Federation of Federal Agency for Science and Innovation (MD-3571.2008.7). The authors thank Drs Mikhail Kozhuhov and Valery Panfilov from the Kursk Regional Clinical Hospital for their assistance in assembling patients with asthma, as well as for their help in diagnosis of the disease.

SAPALDIA

Research support: the Swiss National Science Foundation (grants no 33CSCO-108796, 3247BO-104283, 3247BO-104288, 3247BO-104284, 3247-065896, 3100-059302, 3200-052720, 3200-042532, 4026-028099), the Federal Office for Forest, Environment and Landscape, the Federal Office of Public Health, the Federal Office of Roads and Transport, the canton's government of Aargau, Basel-Stadt, Basel-Land, Geneva, Luzern, Ticino, Valais, and Zürich, the Swiss Lung League, the canton's Lung League of Basel Stadt/ Basel Landschaft, Geneva, Ticino, Valais and Zurich, SUVA, Freiwillige Akademische Gesellschaft, UBS Wealth Foundation, Talecris Biotherapeutics GmbH, Abbott Diagnostics, European Commission 018996 (GABRIEL), Wellcome Trust WT 084703MA. None of the funders had any role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Current SAPALDIA Team: Study directorate: T Rochat (p), NM Probst Hensch (e/g), JM Gaspoz (c), N Künzli (e/exp), C Schindler (s). Scientific team: JC Barthélémy (c), W Berger (g), R Bettschart (p), A Bircher (a), O Brändli (p), C Brombach (n), M Brutsche (p), L Burdet (p), M Frey (p), U Frey (pd), MW Gerbase (p), D Gold (e/c/p), E de Groot (c), W Karrer (p), R Keller (p), B Martin (pa), D Miedinger (o), U Neu (exp), L Nicod (p), M Pons (p), F Roche (c), T Rothe (p), E Russi (p), P Schmid-Grendelmeyer

(a), A Schmidt-Trucksäss (pa), A Turk (p), J Schwartz (e), D. Stolz (p), P Straehl (exp), JM Tschopp (p), A von Eckardstein (cc), E Zemp Stutz (e). Scientific team at coordinating centers: M Adam (e/g), E Boes (g), PO Bridevaux (p), D Carballo (c), E Corradi (e), I Curjuric (e), J Dratva (e), A Di Pasquale (s), E Dupuis Lozeron (s), M Germond (s), L Grize (s), D Keidel (s), S Kriemler (pa), A Kumar (g), M Imboden (g), N Maire (s), A Mehta (e), H Phuleria (exp), E Schaffner (s), GA Thun (g) A Ineichen (exp), M Ragettli (e), M Ritter (exp), T Schikowski (e), M Tarantino (s), M Tsai (e), M Wanner (pa) (a) allergology, (c) cardiology, (cc) clinical chemistry, (e) epidemiology, (exp) exposure, (g) genetic and molecular biology, (m) meteorology, (n) nutrition, (o) occupational health, (p) pneumology, (pa) physical activity, (pd) pediatrics, (s) statistics

Acknowledgements: The study could not have been done without the help of the study participants, technical and administrative support and the medical teams and field workers at the local study sites.

Local fieldworkers : Aarau: S Brun, G Giger, M Sperisen, M Stahel, Basel: C Bürli, C Dahler, N Oertli, I Harreh, F Karrer, G Novicic, N Wytttenbacher, Davos: A Saner, P Senn, R Winzeler, Geneva: F Bonfils, B Blicharz, C Landolt, J Rochat, Lugano: S Boccia, E Gehrig, MT Mandia, G Solari, B Viscardi, Montana: AP Bieri, C Darioly, M Maire, Payerne: F Ding, P Danieli A Vonnez, Wald: D Bodmer, E Hochstrasser, R Kunz, C Meier, J Rakic, U Schafroth, A Walder. Administrative staff: C Gabriel, R Gutknecht.

LifeLines Cohort Study

The LifeLines Cohort Study, and generation and management of GWAS genotype data for the LifeLines Cohort Study is supported by the Netherlands Organization of Scientific Research NWO (grant 175.010.2007.006), the Economic Structure Enhancing Fund (FES) of the Dutch government, the Ministry of Economic Affairs, the Ministry of Education, Culture and Science, the Ministry for Health, Welfare and Sports, the Northern Netherlands Collaboration of Provinces (SNN), the Province of Groningen, University Medical Center Groningen, the University of Groningen, Dutch Kidney Foundation and Dutch Diabetes Research Foundation. The authors are grateful to the study participants, the staff from the LifeLines Cohort Study and Medical Biobank Northern Netherlands, and the participating general practitioners.

References

1. Strachan DP, Rudnicka AR, Power C, Shepherd P, Fuller E, Davis A, et al. Lifecourse influences on health among British adults: effects of region of residence in childhood and adulthood. *Int J Epidemiol* 2007; 36(3): 522-31.
2. Moffatt MF, Gut IG, Demenais F, Strachan DP, Bouzigon E, Heath S, et al. A large-scale, consortium-based genomewide association study of asthma. *N Engl J Med* 2010; 363(13): 1211-21.
3. Burney PG, Luczynska C, Chinn S, Jarvis D. The European Community Respiratory Health Survey. *Eur Respir J* 1994; 7(5): 954-60.
4. The European Community Respiratory Health Survey II. *Eur.Respir.J.* 2002; 20(5): 1071-9.
5. Kauffmann F, Dizier MH, Annesi-Maesano I, Bousquet J, Charpin D, Demenais F, et al. EGEA (Epidemiological study on the Genetics and Environment of Asthma, bronchial hyperresponsiveness and atopy)-- descriptive characteristics. *Clin Exp Allergy* 1999; 29 Suppl 4: 17-21.
6. Siroux V, Boudier A, Bousquet J, Bresson JL, Cracowski JL, Ferran J, et al. Phenotypic determinants of uncontrolled asthma. *J Allergy Clin Immunol* 2009; 124(4): 681,7.e3.
7. Polonikov A.V., Ivanov V.P., Solodilova M.A., Kozhuhov M.A., Panfilov V.I. Tobacco smoking, fruit and vegetable intake modify association between -21A>T polymorphism of catalase gene and risk of bronchial asthma. *Journal of Asthma*. 2009; 46(3): P.217-224.
8. Medea Imboden, Emmanuelle Bouzigon, Ivan Curjuric, Adaikalavan Ramasamy, Ashish Kumar, Dana B. Hancock Genome-wide association study of lung function decline in adults with and without asthma. *J Allergy Clin Immunol* 2012; 129(5): 1218–1228
9. Freidin MB, Kobayakova OS, Ogorodova LM, Puzyrev VP. Association of polymorphisms in the human IL4 and IL5 genes with atopic bronchial asthma and severity of the disease. *Comp Funct Genomics* 2003; 4(3): 346-50.
10. Stolk RP, Rosmalen JG, Postma DS, de Boer RA, Navis G, Slaets JP, et al. Universal risk factors for multifactorial diseases: LifeLines: a three-generation population-based study. *Eur J Epidemiol* 2008; 23(1): 67-74.

S2 Table. Annotation of the top SNPs identified in both approaches

Ch	SNP	Effect allele	Closest gene*	Type
Identified in approach 1: meta-analysis of overall interaction effect				
1	rs7513225	A		5KB_upstream_variant
3	rs9758775	C		intergenic_variant
5	rs3853475	C		nc_transcript_variant;
6	rs1106841	C	XPO5	synonymous_codon;NMD_t
6	rs2812719	A	SH3BGRL2	intron_variant
6	rs723981	T	SH3BGRL3	intron_variant
6	rs1883877	A	SH3BGRL4	intron_variant
7	rs2015523	T	ZNF804B	intron_variant
8	rs7816370	A	CSMD1	nc_transcript_variant;
8	rs17601573	C	PSK	intron_variant
9	rs2890993	G	FREM1	nc_transcript_variant;
9	rs17061224	T		
10	rs7906433	T		intergenic_variant
11	rs3818275	C	SLC1A2	intron_variant;5KB_dow
12	rs11047993	A	U4	intergenic_variant
12	rs11047994	A	U4	intergenic_variant
12	rs4578491	A	U4	intergenic_variant
			U4 =spliceosomal RNA	
			RPL39P27 = ribosomal	
12	rs5011804	C	protein L39 pseudogene 27	intergenic_variant
13	rs4884334	G	U7	intergenic_variant
17	rs8071270	T	U6	intergenic_variant
17	rs7226071	G	U6	intergenic_variant
17	rs6501483	G	U6	intergenic_variant
17	rs2367536	C		intergenic_variant
18	rs724676	T	L3MBTL4	intergenic_variant
19	rs618940	G	LSM14A	intergenic_variant
20	rs6072658	C	PTPRT	intron_variant
20	rs10485689	T	PTPRT	intron_variant
Identified in approach 2: meta-analysis of overall genetic effect in exposed				
1	rs4926457	C		nc_transcript_variant;
1	rs10924824	G	SCCPDH	500B_downstream_varian
1	rs4244627	G		nc_transcript_variant;

1	rs10924823	T	SCCPDH	500B_downstream_varian
2	rs1448187	T		nc_transcript_variant;
2	rs2195614	A		intergenic_variant
2	rs2217431	A		intergenic_variant
2	rs13000320	C		intergenic_variant
3	rs428834	T		intergenic_variant
5	rs6863550	A		intergenic_variant
6	rs943801	C	PDE10A;PDE10	intron_variant;5KB_ups
6	rs2987296	T	PDE10A;PDE10	intron_variant
6	rs643066	T	PDE10A;PDE11	intron_variant
9	rs2988576	A	U2	intergenic_variant
9	rs9969775	A		intergenic_variant
9	rs4338205	A	SH3GL2	intron_variant
9	rs4745437	C		intergenic_variant
9	rs1328550	C		intergenic_variant
10	rs7074731	C	U6	intergenic_variant
12	rs999481	G		500B_downstream_varian
12	rs1716466	G		nc_transcript_variant;
12	rs7954580	A	TMEM132D	intron_variant
13	rs9591994	C	U7	intergenic_variant
13	rs9544173	G		intergenic_variant
16	rs8047401	T	CDY	nc_transcript_variant;
17	rs11077501	C		intergenic_variant
20	rs1984399	A	PTPRT	intron_variant
20	rs727336	T	PTPRT	intron_variant
22	rs4553919	T	SEX6L	intron_variant

Identified in approach 1 and 2: meta-analysis of overall interaction effect

5	rs4912832	A		intergenic_variant
5	rs4541689	G		intergenic_variant
19	rs1759092	G	LSM14A	intron_variant
20	rs7262414	A	PTPRT	intron_variant

* Closest gene within range of 500kb of the position of the SNP.



PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	NA – the term Consortium already implies a meta-analysis
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	page 3
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	NA
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	NA – study details are given in file S1
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	NA
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	NA
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	NA
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	NA
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	page 5: Subjects
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	NA
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	NA
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	NA
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	page 5 and 6 and tables



PRISMA 2009 Checklist

Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	page 6 and 7
----------------------	----	---	--------------

Page 1 of 2

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	NA
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	page 6 and 7
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	NA
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	table 1 for descriptives
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	NA – file S1 for study details
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	supplementary tables, figures 2, 3, and 4
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	Tables 2, 3, 4, and 5
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	NA
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	table 5
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	page 18
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	page 19-20
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	page 18-20
FUNDING			



PRISMA 2009 Checklist

Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	File S1
---------	----	--	---------

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

For more information, visit: www.prisma-statement.org.

Meta-analysis on Genetic Association Studies Checklist | PLOS ONE

	Item	Section name and paragraph number within manuscript
	Introduction	
1	Provide a detailed justification for the polymorphism studied; if a single polymorphism was analyzed, give details as to why others were not included in the meta-analysis.	NA- no candidate gene study or other SNP selection. All available SNPs were studied in a GWA study
2	Provide a detailed justification for the population(s) and clinical condition studied.	Methods - Subjects Introduction
	Methods	
3	Provide full details of the search strategy employed; outline the full electronic search strategy –specific combination of keywords and any limits applied- for at least one database. Specify whether synonyms of polymorphisms/genes (e.g. SNP number) were searched.	NA - The study is not a systematic review but includes all studies with data on adult onset asthma within the Gabriel Consortium
4	Report full details on the inclusion and exclusion criteria applied for selecting studies. <i>Please list the excluded articles and the reasons for exclusion of each article in a supplementary file.</i>	Methods - subjects
5	Provide details on how the quality of the studies included in the analyses was assessed.	All data were collected and analyzed by the 6 individual studies (File S1 and ref 15), QC of genotyping in methods - genotyping and quality control
6	Describe steps taken to contact study authors to identify additional studies and to request missing data.	NA - all studies were part of the GABRIEL consortium
7	Describe how environmental effects were adjusted for, if this adjustment was not conducted, outline the reasons for this.	Methods - statistical analyses
8	Describe the methods of handling heterogeneity/between-study variance.	Methods - statistical analyses
9	Describe how the Hardy-Weinberg equilibrium and linkage disequilibrium were assessed.	HWE: Methods - genotyping and quality control. LD: NA

10	Describe and justify the choice of model for the analyses (per-allele vs per-genotype vs genetic model-free, random effects vs fixed effects).	Methods - statistical analyses
11	Describe whether a sensitivity analysis has been completed.	NA
12	Describe whether an assessment of the effects of population stratification has been conducted.	NA - only adjustment for informative principal components for within-Europe diversity (methods - statistical analyses)
13	Describe whether study-specific results have been assessed and if so the reasons for this (e.g. forest plot).	Methods - statistical analyses. Forest plots in results - figure 1, 2, and 3
	Results	
14	Include flow diagram for the studies included in the meta-analysis as the first figure for the manuscript	NA - of the 23 studies in Gabriel we included 6 based on the availability of data on adult onset asthma. No other selection was applied. It is not necessary to show this in a figure
15	Report details on allele/genotype prevalence.	Results - table 2, 3, and 4
16	Report the effect size estimates and p values for each analysis.	Results - table 2, 3, 4, and 5
	Discussion	
17	Discuss the limitations of the meta-analysis, including genotyping errors/bias and publication bias.	NA - all studies were selected before the study-specific analyses and the meta-analyses were performed. All 6 studies were included in the meta-analysis.
18	If the meta-analysis identifies an association within a subgroup of the population studied but not another, discuss the implications of these results, and if applicable the possibility of subgroup-specific publication bias.	Results - table 5. Publication bias: NA
19	Discuss the suitability of the sample size employed to the research question and the power of the study.	Discussion - 4th and 7th paragraph

