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-analysis[2]. 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 ≥ 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.

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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 Korobaeff (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.

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Kursk State Medical University (KSMU)

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SAPALDIA

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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

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LifeLines Cohort Study

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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

PRISMA 2009 Checklist

Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	page 5 and 6 and tables
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

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

PRISMA 2009 Checklist

FUNDING			
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

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. *Please list the excluded articles and the reasons for exclusion of each article in a supplementary file.*	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 meat-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