Prenatal and prepubertal exposures to tobacco smoke in men may cause lower lung function in future offspring: a three-generation study using a causal modelling approach

Simone Accordini[1,*,†], Lucia Calciano[1,†], Ane Johannessen[2], Bryndis Benediktsdóttir[3], Randi Jacobsen Bertelsen[4,5], Lennart Bråbäck[6], Shyamali C Dharmage[7], Bertil Forsberg[6], Francisco Gómez Real[4,8], John W Holloway[9], Mathias Holm[10], Christer Janson[11], Nils O Jõgi[4,12], Rain Jõgi[12], Andrei Malinovschi[13], Alessandro Marcon[1], Jesús Martínez-Moratalla Rovira[14], José Luis Sánchez-Ramos[15], Vivi Schlünssen[16], Kjell Torén[10], Deborah Jarvis[17,18,‡], Cecilie Svanes[2,‡]

*Corresponding author; †Equal contribution as first authors; ‡Equal contribution as last authors; [1]Unit of Epidemiology and Medical Statistics, Department of Diagnostics and Public Health, University of Verona, Verona, Italy; [2]Centre for International Health, Department of Global Public Health and Primary Care, University of Bergen, Bergen, Norway; [3]Faculty of Medicine, University of Iceland, Reykjavik, Iceland; [4]Department of Clinical Science, University of Bergen, Bergen, Norway; [5]Oral Health Centre of Expertise in Western Norway/Vestland, Bergen, Norway; [6]Section of Sustainable Health, Department of Public Health and Clinical Medicine, Umeå University, Umeå, Sweden; [7]Allergy and Lung Health Unit, School of Population and Global Health, University of Melbourne, Melbourne, Australia; [8]Department of Obstetrics and Gynecology, Haukeland University Hospital, Bergen, Norway; [9]Human Development and Health, Faculty of Medicine, University of Southampton, Southampton, United Kingdom; [10]Occupational and Environmental Medicine, School of Public Health and Community Medicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden; [11]Department of Medical Sciences: Respiratory, Allergy and Sleep Research, Uppsala University, Uppsala, Sweden; [12]Lung Clinic, Tartu University Hospital, Tartu, Estonia; [13]Department of Medical Sciences: Clinical Physiology, Uppsala University, Uppsala, Sweden; [14]Servicio de Neumología del Complejo Hospitalario

Universitario de Albacete (CHUA), Servicio de Salud de Castilla-La Mancha (SESCAM), Albacete, Spain; [15]Department of Nursing, University of Huelva, Huelva, Spain; [16]Department of Public Health, Aarhus University, Aarhus, Denmark; [17]Faculty of Medicine, National Heart & Lung Institute, Imperial College, London, United Kingdom; [18]MRC-PHE Centre for Environment and Health, Imperial College, London, United Kingdom.

CORRESPONDING AUTHOR: Professor Simone Accordini, Sezione di Epidemiologia & Statistica Medica, Dipartimento di Diagnostica e Sanità Pubblica, Università degli Studi di Verona c/o Istituti Biologici II, Strada Le Grazie 8, 37134 Verona, Italy; phone: +39 045 8027657; e-mail: simone.accordini@univr.it; ORCID iD: 0000-0003-1510-6193.

Words count: 3,816

ABSTRACT

Words count: 250

Mechanistic research suggests that lifestyle and environmental factors impact respiratory health across generations by epigenetic changes transmitted through male germ cells. Evidence from studies on humans is very limited.

We investigated multi-generation causal associations to estimate the causal effects of tobacco smoking on lung function within the paternal line. We analysed data from 383 adult offspring (age: 18-47; female: 52.0%) and their 274 fathers, who had participated in the ECRHS/RHINESSA generation study and had provided valid measures of pre-bronchodilator lung function. Two counterfactual-based, multi-level mediation models were developed with: paternal grandmothers' smoking in pregnancy and fathers' smoking initiation in prepuberty as exposures; fathers' FEV_1 and FVC, or FEV_1/FVC z-scores as potential mediators (proxies of unobserved biological mechanisms that are true mediators); offspring's FEV_1 and FVC, or FEV_1/FVC z-scores as outcomes. All effects were summarised as differences in expected z-scores related to fathers' and grandmothers' smoking history.

Fathers' smoking initiation in prepuberty had a negative direct effect on both offspring's FEV_1 (-0.36; 95% confidence interval: -0.63, -0.10) and FVC (-0.50; -0.80, -0.20) compared to fathers' never smoking. Paternal grandmothers' smoking in pregnancy had a negative direct effect on fathers' FEV_1/FVC (-0.57; -1.09, -0.05) and a negative indirect effect on offspring's FEV_1/FVC (-0.12; -0.21, -0.03) compared to grandmothers' not smoking before fathers' birth nor during fathers' childhood.

Fathers' smoking in prepuberty and paternal grandmothers' smoking in pregnancy may cause lower lung function in offspring. Our results support the concept that lifestyle-related exposures during these susceptibility periods influence the health of future generations.

TAKE HOME MESSAGE

Fathers' prepuberty and paternal grandmothers' pregnancy are vulnerable periods to the adverse effects of smoking on offspring's lung function. Preventing smoking in these susceptibility time windows might improve the next generation's health.

KEY WORDS: lung function; tobacco smoking; prepuberty; pregnancy; paternal line; causal inference; multi-level mediation modelling; ALEC; ECRHS; RHINESSA.

INTRODUCTION

Asthma and chronic obstructive pulmonary disease (COPD) are major health challenges across the world [1, 2]. Lifestyle and environmental exposures are important risk factors for these diseases [3, 4], not least when exposure occurs in early life. Emerging evidence suggests that the environment before conception may impair respiratory health of the offspring [5, 6], as supported by animal research [7–9]. In line with this hypothesis, we previously found negative associations between fathers' smoking before 15 years of age (a period compatible with prepuberty, as 15 years is the mean age of completed puberty in boys [10]) and their offspring's asthma phenotypes [11, 12]. We also identified the same susceptibility time window for fathers' overweight [13].

Studies on human health [14], including respiratory diseases [15–18], are currently more focused on epigenetics. Heritable modification of DNA methylation may be a key mechanism for the effects of preconception and early life exposures on disease phenotypes in subsequent generations. Foetal life and prepuberty are time windows with a higher susceptibility to environmental exposures through their impact on epigenetic programming of the male germ cell line [19, 20]. These vulnerable periods may represent windows of opportunity for preventive interventions in males aimed at improving the health of current and future generations.

Causal statistical inference [21] can contribute to shed light on the mechanisms involved in the pathways from exposures in different generations and susceptibility periods, to health outcomes in subsequent generations. A multiple-exposure, multiple-mediator, multiple-outcome (MEMMMO) framework has recently been proposed for the identification of complex multi-generation effects [22]. In line with this new perspective, we investigated causal associations to estimate the causal effects of fathers' smoking initiation in prepuberty (generation G1) and grandmothers' smoking in pregnancy (generation G0) on offspring's lung function (generation G2) within the paternal line. As part of the Ageing Lungs in European Cohorts (ALEC) Study (*www.alecstudy.org*), we analysed

data from an ongoing survey on respiratory health in adults (European Community Respiratory Health Survey, ECRHS; *www.ecrhs.org*) and its extension to the generation of their offspring (Respiratory Health in Northern Europe, Spain and Australia, RHINESSA; *www.rhinessa.net*).

METHODS

Study design

ECRHS is an international, population-based, cohort study on respiratory health in subjects aged 20-44 at the time of recruitment (ECRHS I; 1991-1993) [23]. At baseline, each participant was sent a brief screening questionnaire (stage 1) and, from those who responded, a 20% "*random sample*" was invited to undergo a more detailed clinical examination (stage 2). An additional "*symptomatic sample*" of adults with asthma-like symptoms was also recruited at stage 2. The follow-up of the participants in stage 2 took place in 1998-2002 (ECRHS II) [24] and in 2010-2013 (ECRHS III) [25]. These subjects underwent a standardized clinical interview, lung function and laboratory tests on all the occasions. RHINESSA is an international study on health and disease in the offspring of the ECRHS participants in ten centres from Northern Europe, Spain, and Australia [13]. Extensive questionnaire and lung function data were collected in 2013-2016, based on protocols adapted to those used in the ECRHS. Written informed consent was obtained from each participant in the ECRHS/RHINESSA studies. Ethics approval was obtained by the appropriate ethics committee in each centre (see *helse-bergen.no/seksjon/RHINESSA/Documents/Ethic%20Committees%20list.pdf* and the online supplementary material).

Lung function and definitions

Maximum pre-bronchodilator forced expiratory volume in one second (FEV_1) and forced vital capacity (FVC) from at least two technically satisfactory manoeuvres were measured according to the American Thoracic Society (ATS) criteria for repeatability [26], as part of the ECRHS and RHINESSA clinical examinations. Post-bronchodilator spirometry was also measured in ECRHS at the last follow-up contact (ECRHS III) and in RHINESSA. In the present analyses, we used the fathers' lung function measurements at baseline (ECRHS I) or at the first available occasion

(ECRHS II or ECRHS III) if baseline spirometry had not been performed or had not fulfilled the ATS criteria. The Global Lung function Initiative (GLI) z-scores [27] were calculated by using the European Respiratory Society freeware software (*www.ers-education.org/guidelines/global-lung-function-initiative/spirometry-tools/desktop-sheet-calculator.aspx*), in order to control for the dependency of lung function on sex, age and height.

The fathers provided detailed information on their own smoking history, including when they had started smoking, during interviews at each ECRHS examination. Fathers' smoking was categorized as "*smoking initiation before 15 years of age*" (i.e. during prepuberty), "*smoking initiation at 15 years of age or older*" or "*never smoking*". Fathers' education level was self-reported at ECRHS I and was considered "*low*" if less than or equal to the minimum school-leaving age in their country before the start of the ECRHS study [28]. Fathers' occupational class was derived from the longest-held job during the follow-up period between ECRHS I and II, with categories based on the major group classification in the International Standard Classification of Occupations (ISCO) [29]. At ECRHS I, the fathers reported whether their mother had smoked before or after their birth. Consequently, grandmothers' smoking was classified as "*smoking when the father was in utero*", "*smoking before pregnancy or during father's childhood (or unknown smoking period)*" or "*not smoking before father's birth nor during father's childhood*". At ECRHS III, the fathers provided information on their parents' education level (defined "*low*" if both grandparents were reported to have only studied up to the minimum school-leaving age). In RHINESSA, the offspring reported their own smoking history and education level (defined "*low*" if a subject had only studied up to the minimum school-leaving age), and whether their mother had smoked before or after their birth, during interviews with similarly worded questions to the ECRHS.

Study subjects

Of the 2,302 males from Estonia (Tartu), Norway (Bergen), Spain (Albacete, Huelva) and Sweden (Gothenburg, Umea, Uppsala) who had undergone a clinical examination in ECRHS I-III, 1,241 participated in ECRHS III. Of these, 913 men reported having at least one offspring and 297 of them had at least one adult offspring who participated in the RHINESSA clinical stage and were thus eligible for inclusion in the present analyses (figure 1). These fathers had valid lung function measurements and reported complete information on their own and their mother's smoking history. Of the 420 adult offspring who had originated from these 297 fathers, 383 individuals had valid lung function measurements. These offspring and their 274 fathers (92.7% from the ECRHS "*random sample*") were included in our study.

Statistical analyses

Counterfactual-based mediation analyses [30] within a hierarchical framework were carried out to investigate the pathways among grandmothers' and fathers' tobacco smoking in vulnerable periods and offspring's lung function within the paternal line. Our data have a hierarchical structure because the offspring siblings (level 1 units) have the same biological father (level 2 units) and because their fathers were sampled from different centres (level 3 units). The total effect of each exposure on each outcome was decomposed into its natural direct effect (i.e. the effect of the exposure on the outcome via a pathway that does not involve the mediator) and its natural indirect (mediated) effect (i.e. the effect of the exposure on the outcome due to the effect of the exposure on the mediator) [31]. The main requirement for mediation is that the indirect effect is statistically significant [32], when the observed mediated effect is robust to potential confounding by some unmeasured variable [33].

Two multi-level mediation models were used within the paternal line. *Model 1* and *model 2* were aimed at estimating the multi-generation effects of smoking on pre-bronchodilator FEV_1 and FVC

(figure 2), or FEV_1/FVC (figure 3), respectively. *Model 1* included fathers' FEV_1 and FVC z-scores as the normally distributed, correlated, parallel mediators, and offspring's FEV_1 and FVC z-scores as the normally distributed, correlated, parallel outcomes. *Model 2* included fathers' FEV_1/FVC z-score as the normally distributed mediator and offspring's FEV_1/FVC z-score as the normally distributed outcome. Each lung function variable in fathers (measured in adulthood) was considered a proxy of unobserved biological mechanisms that are true mediators. Latent mediators were not included in the models as we had a single indicator for each mediator, and using single-indicator latent variables often causes identification problems in model specification [34]. In both models, grandmothers' and fathers' smoking were the exposures of interest, and grandparents' and fathers' education level, and fathers' occupational class were analysed as potential confounders. We verified whether these potential confounders represent the "*minimal sufficient adjustment set*" (i.e. the smallest set of measured covariates that needs to be included in order to eliminate confounding), by using directed acyclic graphs (DAGs) [35] in DAGitty (*dagitty.net*; see the online supplementary material). In addition, fathers' age, mothers' smoking before or after offspring's birth, and offspring's sex, age, education level and smoking were analysed as adjusting variables of the exposure-mediator-outcome relationships.

Model 1 and *model 2* had random intercept terms at level 2 (father), cluster-robust standard errors (country = cluster variable) and a "*2→2→1*" configuration [36] [i.e. the exposures and the mediators were measured at level 2 (father), whereas the outcomes were measured at level 1 (offspring)]. The natural (counterfactual-based) direct and indirect effects of the exposures (grandmothers' and fathers' smoking) on the normally distributed mediators (fathers' z-scores) and outcomes (offspring's z-scores) were summarised as differences (Δ) in expected z-scores. The natural direct effect is offspring's Δz-score for the change in exposure status, keeping fathers' z-score at its expected value when the exposure is absent. The natural indirect effect is offspring's Δz-score when the exposure is present, but fathers' z-score changes from its expected value when the

exposure is absent to its expected value when the exposure is present. The magnitude of the direct and indirect effects, and their 95% confidence interval (95%CI) were computed based on the maximum likelihood estimator with robust standard errors [36] and the distribution-of-the-product method [37], respectively.

STATA 15 (StataCorp, College Station, TX), Mplus 8 (Muthén & Muthén, Los Angeles, CA), and R 3.6.1 (*www.R-project.org*) were used for the statistical analyses.

Sensitivity analyses

Sensitivity analyses were performed in order to assess:

- whether the estimated effects changed after the inclusion of up to two unmeasured confounders (U_1 and U_2) in *model 1* and *model 2*, by using probabilistic (Monte Carlo) simulations in the Umediation package [38] (*github.com/SharonLutz/Umediation*; see the online supplementary material);
- which is the minimum strength of association that an unmeasured confounder would need to have with both the mediator and outcome, conditional on the measured confounders, to fully explain away the observed direct or indirect effects (mediational E-values) [39], by using the Evalue package [40] (*github.com/mayamathur/evalue_package*). The mediational E-values were returned on the risk ratio (RR) scale through an approximate conversion [41, 42];
- whether the estimated effects of fathers' smoking in prepuberty changed when the analyses were repeated by excluding the 182 offspring whose father had smoked after their birth;
- whether the estimated effects changed when using offspring's post-bronchodilator lung function measurements (available from 369 offspring).

RESULTS

Main characteristics of the study subjects

At the time of lung function assessment, the median age of the 274 fathers and their 383 offspring (female: 52.0%) was 37 and 28 years, respectively (table 1 and table 2). In our sample, 10.2%, 8.4%, and 7.7% of the fathers had FEV_1, FVC or FEV_1/FVC z-scores below -1.645 (fifth percentile), respectively, whereas these figures were 6.0%, 3.7% and 6.8% for their offspring. Among the fathers, 9.1% had started smoking before 15 years of age (i.e. during prepuberty) and 7.7% reported that their mother (grandmothers in the present analyses) had smoked during their pregnancy. Among the offspring, the percentage of ever smokers was 30.3% and 50.7% reported that their mother had smoked before or after their birth.

Fathers' smoking initiation in prepuberty

Fathers' smoking initiation in prepuberty (generation G1) had a negative direct effect on their own FEV_1/FVC [Δz-score (95%CI): -0.36 (-0.68, -0.04)] compared to fathers' never smoking (table 3). This exposure had a negative direct effect on both offspring's FEV_1 [-0.36 (-0.63, -0.10)] and FVC [-0.50 (-0.80, -0.20)] (generation G2), but we did not observe a statistically significant effect on offspring's FEV_1/FVC. No mediated effect of fathers' smoking in prepuberty on offspring's pulmonary values was identified (table 4) (*direct-only non-mediation*). Fathers' smoking initiation at later ages had a negative direct effect on their own FEV_1 [-0.27 (-0.51, -0.02)] and FEV_1/FVC [-0.20 (-0.37, -0.04)], but we did not find an effect on offspring's lung function.

Grandmothers' smoking in pregnancy

Grandmothers' smoking when the father was *in utero* (generation G0) had a negative direct effect on fathers' FEV_1/FVC (generation G1) [-0.57 (-1.09, -0.05)] compared to grandmothers' not

smoking before fathers' birth nor during fathers' childhood (table 3). Grandmothers' smoking while pregnant with the father had no direct effect on grandchildren's lung function (generation G2). This exposure had a negative mediated effect, through unobserved biological mechanisms for which fathers' FEV_1/FVC in adulthood is an indicator, on grandchildren's FEV_1/FVC [-0.12 (-0.21, -0.03)] (table 4) (*indirect-only mediation*).

Sensitivity analyses

Simulations suggested that unmeasured confounding could reasonably be assumed to have a low impact on the effects of fathers' and grandmothers' smoking (supplementary Figure S4 and Figure S5). When the effect (beta regression coefficient) of each unmeasured confounder (U_1 and U_2) on the exposure, mediator and outcome was set less than or equal to five, the proportion of simulations where the results matched (whether U_1 and U_2 were included or excluded from the models) was greater than 72.8% and the average absolute difference of the average effects was lower than 0.083. U_1 and U_2 changed the results for very strong effects only (i.e. for beta regression coefficients greater than five).

The mediational E-value for the natural direct effect of father's smoking in prepuberty on offspring's FEV_1 and FVC was 2.31 and 2.85, respectively, whereas the mediational E-value for the natural indirect effect of grandmother's smoking in pregnancy on offspring's FEV_1/FVC was 1.50. Therefore, an unmeasured confounder must be associated with both the mediator and outcome with an approximate RR at least equal to 2.31, 2.85 or 1.50 (when the outcome is FEV_1, FVC or FEV_1/FVC, respectively) to completely explain away the observed effects.

Exclusion of the offspring of fathers who had smoked after their birth provided stronger estimates of the direct effect of fathers' smoking in prepuberty on offspring's FEV_1 [-0.55 (-1.18, 0.07)] and FVC [-0.77 (-1.61, 0.07)] than that obtained in the main analysis. However, these estimates did not

reach statistical significance because of data sparseness, as only 8 offspring were born to 7 fathers who had started smoking in prepuberty and had quit smoking before their birth.

When offspring's post-bronchodilator values were used, the direct effect of fathers' smoking on offspring's FVC [-0.35 (-0.62, -0.07)] and the indirect effect of grandmothers' smoking on grandchildren's FEV_1/FVC [-0.09 (-0.17, -0.01)] were reduced. However, the direct effect of fathers' smoking on offspring's FEV_1 did not reach statistical significance [-0.19 (-0.48, 0.11)].

DISCUSSION

In the present study, we found that fathers' and paternal grandmothers' smoking in vulnerable time windows may negatively affect lung function in the next two generations. These results were obtained using a method of causal inference with observational data and were strengthened by sensitivity analyses accounting for unmeasured confounding. Our novel observation is that the offspring of fathers who had smoked during prepuberty may have lower FEV_1 and FVC values (suggesting an effect on reducing the overall lung volumes), compared to the offspring of fathers who had never smoked. Another key result is the negative indirect effect of grandmothers' smoking when the father was *in utero* on grandchildren's FEV_1/FVC. This finding suggests that smoking during pregnancy (generation G0) may not only increase the risk of airflow obstruction in generation G1, but it may also have a negative effect on generation G2 within the paternal line. Our results support the concept that lifestyle-related exposures during prepuberty in males and during pregnancy influence the health of future generations.

Fathers' smoking initiation in prepuberty

In the present analyses, the effect of fathers' prepubertal smoking on offspring's FEV_1 and FVC was observed regardless of the effect of mothers' smoking before or after offspring's birth, and it was even present after the exclusion of the offspring of fathers who had smoked after their birth. When offspring's post-bronchodilator values were used, the effect on FVC was reduced but it was still statistically significant. Moreover, fathers' smoking initiation at later ages had no effect on their offspring's lung function.

Unmeasured genetic confounding unlikely explains away the direct effect of paternal early smoking on offspring's FEV_1 and FVC, as the few risk loci for both nicotine dependence and lung function identified in genetic studies (such as rs16969968 in CHRNA5 gene) [43, 44] have weaker

associations than the computed mediational E-values [39]. Therefore, we speculate that our findings could reflect epigenetic alterations (such as DNA methylation, histone modification and microRNAs) in developing germ cells [45-47] leading to reduced lung volumes in offspring. The heritable effects of smoking in young men seem to be biologically plausible, because male prepuberty represents a critical period for the germ line development [19, 20], which might give higher susceptibility to tobacco-related effects on the epigenetic profile of gametes. In addition, the observed negative impact of fathers' prepubertal smoking on offspring's FEV_1 and FVC may reflect the direct toxicogenic effects of cigarette smoke on biological processes involved in metabolic health. Prepubertal start of father's smoking may contribute to obesity in adolescent [48] and adult sons [49], and obese adults show a spirometry pattern characterised by lower FEV_1, FVC, total lung capacity and residual volume [50].

Our results are supported by findings from previous analyses of the same population, in which we observed that fathers' smoking initiation in prepuberty was associated with an increased risk for non-allergic asthma in offspring [11, 12]. In addition, we found that fathers' overweight onset before 15 years of age had a direct effect on non-allergic asthma in the next generation [13]. All these results strengthen the hypothesis that life-style and environmental exposures during male prepuberty may affect the respiratory health of future offspring. Our current findings may have considerable public health implications, particularly as tobacco smoking in 11- to 15-year-old boys has increased in different European regions over recent decades [51], as well as the use of moist oral tobacco and e-cigarettes among the very young. Nevertheless, we acknowledge that early smoking in men could also be a marker for other causative factors, as risky behaviours of several kinds can be associated with smoking in adolescents.

Grandmothers' smoking in pregnancy

Our study highlights that grandmothers' smoking in pregnancy may negatively affect FEV_1/FVC in the next two generations within the paternal line. Accordingly, findings from the UK Biobank indicate an excess reduction in FEV_1/FVC among sons due to maternal smoking around delivery, whereas the reduction in FVC is very modest [52]. The negative direct effect of this prenatal exposure on fathers' lung function is supported by previous evidence in humans and animals [53], because nicotine can penetrate the placental barriers and adversely affect lung development [54]. In addition, maternal smoking during pregnancy is associated with low birth weight and preterm delivery [55, 56], which are linked to reduced lung function in adulthood [57, 58].

We speculate that the negative indirect effect of grandmothers' smoking during pregnancy (through biological mechanisms for which fathers' lung function in adulthood is an indicator) on grandchildren's FEV_1/FVC could be explained by epigenetic changes [59, 60], resulting in airflow obstruction in offspring [60], and this effect is unlikely fully explained away by unmeasured risk loci, as suggested by the computed mediational E-value [39]. Tobacco smoking may cause heritable changes of the germ cell epigenome, particularly in the prenatal period [60, 61]. An animal model of multi-generation nicotine-induced asthma showed that epigenetic modifications can affect lung function in second-generation offspring [62]. Furthermore, a study on humans highlighted a link between prenatal exposure to tobacco smoke, DNA methylation changes and asthma-related lung function [63].

Strengths and weaknesses

Epidemiological studies on the early life origins of diseases usually focus on mothers' exposures immediately before conception and during pregnancy. By contrast, RHINESSA included the offspring of both male and female participants in ECRHS, and the ECRHS survey followed adult men and women through their reproductive age. Thus, the ECRHS/RHINESSA studies collected

extensive data on exposures occurring well in advance before conception (together with objective measurements of lung function) in two generations within both the paternal and maternal lines. Another major strength of the present analyses is the statistical approach used for assessing causal associations to estimate causal effects across generations. We used mediation models [30], which have become increasingly relevant for causal inference in epidemiological studies, in order to investigate complex pathways among multiple exposures, multiple mediators and multiple outcomes (MEMMMO framework) [22]. Finally, our results were supported by: (i) probabilistic simulations on the impact of unmeasured confounding [38]; (ii) mediational E-values [39] to quantify robustness to unmeasured confounding, e.g. due to risk loci for both nicotine dependence and lung function [43, 44]; (iii) a subgroup analysis to strengthen the causal interpretation of the observed effects of fathers' smoking in prepuberty on lung function in the next generation; (iv) a sensitivity analysis based on offspring's post-bronchodilator spirometry, which is required to identify irreversible airflow obstruction [2].

The main limitation of our analyses is that parental information from the ECRHS study was available for one parent only. The information on mothers and paternal grandparents was offspring- and father-reported, respectively, rather than directly assessed, generating a possible information bias. However, validation studies from our cohorts on tobacco smoking [64] and asthma [65] reports across generations suggested that, although the recall bias is present, it is likely to have a limited effect. Secondly, post-bronchodilator spirometry was not used in the main analyses because it was available for a subset of the ECRHS fathers only. Thirdly, the number of exposed subjects was small. Due to this data sparseness, we could not assess the moderating effects of offspring's sex [66] and mothers' smoking before or after offspring's birth [5] (i.e. specific patterns of effects where grandmothers' and fathers' risk factors differently affect offspring according to their sex or their exposure to mothers' smoking). Lastly, it is possible that unmeasured confounders (such as

genetic, other lifestyle and environmental factors in the three generations) may be important. In particular, we could not control for the potential confounding effect of genetic factors in our models because polymorphisms were available for a subset of the fathers and for offspring only, whereas genotypes on grandmothers, grandfathers and mothers are also needed to separate the influence of genetic inheritance from the effect of lifestyle/environmental exposures [67]. However, we estimated that unmeasured confounding had a limited impact on the effects identified in our analyses.

Conclusions

Men who initiated smoking before 15 years of age may have offspring with lower lung function compared to men who had never smoked. Grandmothers' smoking in pregnancy may have a negative impact on their sons' lung function, an effect that could be carried over to their grandchildren. These results support the concept that lifestyle-related exposures in male prepuberty and in pregnancy influence the health of future generations. Preventing smoking in these susceptibility time windows might have potential benefits for several generations.

ACKNOWLEDGEMENTS

The present analyses are part of the Research Council of Norway FRIPRO project (led by Cecilie Svanes) and the ALEC Study (*www.alecstudy.org*; led by Deborah Jarvis). The present manuscript contributes to ALEC Workpackage 2 (led by Cecilie Svanes). The RHINESSA PIs and vice-PIs are listed at *helse-bergen.no/seksjon/RHINESSA/Documents/Rhinessa PI and vice PI.pdf*. The ECRHS PIs and team members are reported in the online supplementary material.

SUPPORT STATEMENT

The ALEC Study received funding from the European Union's Horizon 2020 research and innovation programme (Grant No. 633212). RHINESSA received funding from the Research Council of Norway (Grants No. 214123, 228174 and 274767), the Bergen Medical Research Foundation, the Western Norwegian Regional Health Authorities (Grants No. 911631, 911892 and 912011), the Norwegian Labour Inspection, the Norwegian Asthma and Allergy Association, the Danish Wood Foundation (Grant No. 444508795), the Danish Working Environment Authority (Grant No. 20150067134), the Swedish Lung Foundation, the Swedish Asthma and Allergy Association, and the Estonian Research Council (Grant No. PUT562). Both the ALEC Study and RHINESSA received funding from the Australian NHMRC. The co-ordination of ECRHS was supported by the European Commission (ECRHS I and II) and the Medical Research Council (ECRHS III). Local funding agencies for ECRHS are reported in the online supplementary material. The funders had no role in the study design, data collection, analysis and interpretation, or writing of the report. The corresponding author had full access to all data and had final responsibility for the decision to submit for publication.

CONFLICT OF INTEREST

J.W. Holloway reports grants from the European Union's Horizon 2020 research and innovation programme and the Research Council of Norway, during the conduct of the study. R. Jõgi reports grants from the Estonian Research Council (Personal Research Grant No. 562) during the conduct of the study and personal fees for consultancy and payment for lectures from Boehringer Ingelheim, GSK and Novartis. D. Jarvis reports grants from the European Union during the conduct of the study. All other authors declare no competing interests.

REFERENCES

1 Global Initiative for Asthma. NHLBI/WHO Workshop Report: Global Strategy for Asthma Management and Prevention. Bethesda, National Institutes of Health Publication No. 02-3659, 1995. Revised 2018.

2 Global Initiative for Chronic Obstructive Lung Disease. Global Strategy for the Diagnosis, Management, and Prevention of Chronic Obstructive Pulmonary Disease. NHLBI/WHO Workshop Report. Bethesda, National Institutes of Health Publication No. 2701, 2001. Revised 2018.

3 Beasley R, Semprini A, Mitchell EA. Risk factors for asthma: is prevention possible? Lancet 2015; 386: 1075-1085.

4 Mannino DM, Buist AS. Global burden of COPD: risk factors, prevalence, and future trends. Lancet 2007; 370: 765-773.

5 Svanes C, Omenaas E, Jarvis D, Chinn S, Gulsvik A, Burney P. Parental smoking in childhood and adult obstructive lung disease: results from the European Community Respiratory Health Survey. Thorax 2004; 59: 295-302.

6 Svanes C, Sunyer J, Plana E, *et al*. Early life origins of chronic obstructive pulmonary disease. Thorax 2010; 65: 14-20.

7 Maritz GS, Mutemwa M. The effect of grand maternal nicotine exposure during gestation and lactation on lung integrity of the F2 generation. Pediatr Pulmonol 2014; 49: 67-75.

8 Mørkve Knudsen T, Rezwan FI, Jiang Y, Karmaus W, Svanes C, Holloway JW. Trans- and inter-generational epigenetic inheritance in allergic diseases. J Allergy Clin Immunol 2018; 142: 765-772.

9 Hammer B, Wagner C, Divac Rankov A, *et al*. In utero exposure to cigarette smoke and effects across generations: A conference of animals on asthma. Clin Exp Allergy 2018; 48: 1378-1390.

10 Håkonsen LB, Olsen J, Støvring H, *et al*. Maternal cigarette smoking during pregnancy and pubertal development in sons. A follow-up study of a birth cohort. Andrology 2013; 1: 348-355.

11 Svanes C, Koplin J, Skulstad SM, *et al*. Father's environment before conception and asthma risk in his children: a multi-generation analysis of the Respiratory Health In Northern Europe study. Int J Epidemiol 2017; 46: 235-245.

12 Accordini S, Calciano L, Johannessen A, *et al*. A three-generation study on the association of tobacco smoking with asthma. Int J Epidemiol 2018; 47: 1106-1117.

13 Johannessen A, Lønnebotn M, Calciano L, *et al*. Being overweight in childhood, puberty, or early adulthood: Changing the asthma risk in the next generation? J Allergy Clin Immunol 2020; 145: 791-799.

14 Petronis A. Epigenetics as a unifying principle in the aetiology of complex traits and diseases. Nature 2010; 465: 721-727.

15 Gibbs K, Collaco JM, McGrath-Morrow SA. Impact of tobacco smoke and nicotine exposure on lung development. Chest 2016; 149: 552-561.

16 Singh SP, Chand HS, Langley RJ, *et al*. Gestational exposure to sidestream (secondhand) cigarette smoke promotes transgenerational epigenetic transmission of exacerbated allergic asthma and bronchopulmonary dysplasia. J Immunol 2017; 198: 3815-3822.

17 Miller RL, Lawrence J. Understanding root causes of asthma: perinatal environmental exposures and epigenetic regulation. Ann Am Thorac Soc 2018; 15(Suppl 2): S103-108.

18 Davidson EJ, Yang IV. Role of epigenetics in the development of childhood asthma. Curr Opin Allergy Clin Immunol 2018; 18: 132-138.

19 Soubry A, Hoyo C, Jirtle RL, Murphy SK. A paternal environmental legacy: evidence for epigenetic inheritance through the male germ line. Bioessays 2014; 36: 359-371.

20 Ly L, Chan D, Trasler JM. Developmental windows of susceptibility for epigenetic inheritance through the male germline. Semin Cell Dev Biol 2015; 43: 96-105.

21 Lederer DJ, Bell SC, Branson RD, *et al*. Control of confounding and reporting of results in causal inference studies. Guidance for authors from editors of respiratory, sleep, and critical care journals. Ann Am Thorac Soc 2019; 16: 22-28.

22 Arah OA. Commentary: Tobacco smoking and asthma: multigenerational effects, epigenetics and multilevel causal mediation analysis. Int J Epidemiol 2018; 47: 1117-1119.

23 Burney PG, Luczynska C, Chinn S, Jarvis D. The European Community Respiratory Health Survey. Eur Respir J 1994; 7: 954-960.

24 European Community Respiratory Health Survey II Steering Committee. The European Community Respiratory Health Survey II. Eur Respir J 2002; 20: 1071-1079.

25 Amaral AFS, Newson RB, Abramson MJ, *et al*. Changes in IgE sensitization and total IgE levels over 20 years of follow-up. J Allergy Clin Immunol 2016; 137: 1788-1795.

26 Miller MR, Hankinson J, Brusasco V, *et al*. Standardisation of spirometry. Eur Respir J 2005; 26: 319-338.

27 Quanjer PH, Stanojevic S, Cole TJ, *et al*; ERS Global Lung function Initiative. Multi-ethnic reference values for spirometry for the 3-95-year age range: the global lung function 2012 equations. Eur Respir J 2012; 40: 1324-1343.

28 Hill M. Social policy: a comparative analysis. London, Prentice-Hall/Harvester Wheatsheaf, 1996.

29 Ellison-Loschmann L, Sunyer J, Plana E, *et al*. Socioeconomic status, asthma and chronic bronchitis in a large community-based study. Eur Respir J 2007; 29: 897-905.

30 VanderWeele TJ. Mediation Analysis: A Practitioner's Guide. Annu Rev Public Health 2016; 37: 17-32.

31 Pearl J. Direct and indirect effects. In: Proceedings of the Seventeenth Conference on Uncertainy in Articial Intelligence. San Francisco, Morgan Kaufmann, 2001; pp. 411-420.

32 Zhao X, Lynch Jr JG, Chen Q. Reconsidering Baron and Kenny: Myths and truths about mediation analysis. J Consum Res 2010; 37: 197-206.

33 Valente MJ, Pelham WE III, Smyth H, MacKinnon DP. Confounding in statistical mediation analysis: What it is and how to address it. J Couns Psychol 2017; 64: 659-671.

34 Kenny DA. Identification: Overview. 2011; available at: *davidakenny.net/cm/identify.htm*.

35 Greenland S, Pearl J, Robins JM. Causal diagrams for epidemiologic research. Epidemiology 1999; 10: 37-48.

36 Muthén BO, Muthén LK, Asparouhov T. Regression and Mediation Analysis Using Mplus. Los Angeles, Muthén & Muthén, 2017.

37 Tofighi D, MacKinnon DP. RMediation: an R package for mediation analysis confidence intervals. Behav Res Methods 2011; 43: 692-700.

38 Lutz SM, Thwing A, Schmiege S, *et al*. Examining the role of unmeasured confounding in mediation analysis with genetic and genomic applications. BMC Bioinformatics 2017; 18: 344.

39 Smith LH, VanderWeele TJ. Mediational E-values: Approximate Sensitivity Analysis for Unmeasured Mediator-Outcome Confounding. Epidemiology 2019; 30: 835-837.

40 Mathur MB, Ding P, Riddell CA, VanderWeele TJ. Web Site and R Package for Computing E-values. Epidemiology 2018; 29: e45-e47.

41 Chinn S. A simple method for converting an odds ratio to effect size for use in meta-analysis. Stat Med 2000; 19: 3127-3131.

42 VanderWeele TJ. On a square-root transformation of the odds ratio for a common outcome. Epidemiology 2017; 28: e58-e60.

43 Saccone SF, Hinrichs AL, Saccone NL, *et al*. Cholinergic nicotinic receptor genes implicated in a nicotine dependence association study targeting 348 candidate genes with 3713 SNPs. Hum Mol Genet 2007; 16: 36-49.

44 Lutz SM, Cho MH, Young K, *et al*. A genome-wide association study identifies risk loci for spirometric measures among smokers of European and African ancestry. BMC Genet 2015; 16: 138.

45 Marczylo EL, Amoako AA, Konje JC, Gant TW, Marczylo TH. Smoking induces differential miRNA expression in human spermatozoa: a potential transgenerational epigenetic concern? Epigenetics 2012; 7: 432-439.

46 Mørkve Knudsen GT, Rezwan FI, Johannessen A, *et al*. Epigenome-wide association of father's smoking with offspring DNA methylation: a hypothesis-generating study. Environ Epigenet 2019; 5: dvz023.

47 Gomez JL. Epigenetics in asthma. Curr Allergy Asthma Rep 2019; 19: 56.

48 Northstone K, Golding J, Davey Smith G, Miller LL, Pembrey M. Prepubertal start of father's smoking and increased body fat in his sons: further characterisation of paternal transgenerational responses. Eur J Hum Genet 2014; 22: 1382-1386.

49 Mørkve Knudsen GT, Dharmage S, Janson C, *et al*. Parents' smoking onset before conception as related to body mass index and fat mass in adult offspring: Findings from the RHINESSA generation study. PLoS One 2020; 15: e0235632.

50 Forno E, Han YY, Mullen J, Celedón JC. Overweight, Obesity, and Lung Function in Children and Adults-A Meta-analysis. J Allergy Clin Immunol Pract 2018; 6: 570-581.e10.

51 Marcon A, Pesce G, Calciano L, *et al*. Trends in smoking initiation in Europe over 40 years: A retrospective cohort study. PLoS One 2018; 13: e0201881.

52 Magnus MC, Henderson J, Tilling K, Howe LD, Fraser A. Independent and combined associations of maternal and own smoking with adult lung function and COPD. Int J Epidemiol 2018: 47; 1855-1864.

53 Maritz GS, Harding R. Life-long programming implications of exposure to tobacco smoking and nicotine before and soon after birth: evidence for altered lung development. Int J Environ Res Public Health 2011; 8: 875-898.

54 Sekhon HS, Keller JA, Benowitz NL, Spindel ER. Prenatal nicotine exposure alters pulmonary function in newborn rhesus monkeys. Am J Respir Crit Care Med 2001; 164: 989-994.

55 Davies DP, Abernethy M. Cigarette smoking in pregnancy: Associations with maternal weight gain and fetal growth. Lancet 1976; 1: 385-387.

56 Shiono PH, Klebanoff MA, Rhoads GG. Smoking and drinking during pregnancy. Their effects on preterm birth. JAMA 1986; 255: 82-84.

57 Cai Y, Shaheen SO, Hardy R, Kuh D, Hansell AL. Birth weight, early childhood growth and lung function in middle to early old age: 1946 British birth cohort. Thorax 2016; 71: 916-922.

58 Näsänen-Gilmore P, Sipola-Leppänen M, Tikanmäki M, *et al*. Lung function in adults born preterm. PLoS One 2018; 13: e0205979.

59 Skinner MK. Endocrine disruptor induction of epigenetic transgenerational inheritance of disease. Mol Cell Endocrinol 2014; 398: 4-12.

60 Arshad SH, Karmaus W, Zhang H, Holloway JW. Multigenerational cohorts in patients with asthma and allergy. J Allergy Clin Immunol 2017; 139: 415-421.

61 Perera F, Herbstman J. Prenatal environmental exposures, epigenetics, and disease. Reprod Toxicol 2011; 31: 363-373.

62 Rehan VK, Liu J, Naeem E, *et al*. Perinatal nicotine exposure induces asthma in second generation offspring. BMC Med 2012; 10: 129.

63 Patil VK, Holloway JW, Zhang H, *et al*. Interaction of prenatal maternal smoking, interleukin 13 genetic variants and DNA methylation influencing airflow and airway reactivity. Clin Epigenetics 2013; 5: 22.

64 Pape K, Svanes C, Malinovschi A, *et al*. Agreement of offspring-reported parental smoking status: the RHINESSA generation study. BMC Public Health 2019; 19: 94.

65 Kuiper IN, Svanes C, Benediktsdottir B, *et al*. Agreement in reporting of asthma by parents or offspring - the RHINESSA generation study. BMC Pulm Med 2018; 18: 122.

66 Pembrey ME, Bygren LO, Kaati G, *et al*. Sex-specific, male-line transgenerational responses in humans. Eur J Hum Genet 2006; 14: 159-166.

67 Richmond RC, Timpson NJ, Felix JF, *et al*. Using genetic variation to explore the causal effect of maternal pregnancy adiposity on future offspring adiposity: a Mendelian randomisation study. PLoS Med 2017; 14: e1002221.

Table 1. Main characteristics of the fathers and father-reported information on their parents (grandparents).

Generation		N° of fathers = 274
Grandparents (G0)	Education level, %	
	low*	31.0
	high	36.9
	unknown	32.1
	Grandmother's smoking, %	
	when the father was *in utero*	7.7
	before pregnancy or during father's childhood (or unknown smoking period)	18.6
	not smoking before father's birth nor during father's childhood	73.7
Father (G1)	Age (years), median (range)	37 (21-63)
	Pre-bronchodilator FEV_1 z-score, mean (sd)	-0.35 (1.09)
	Pre-bronchodilator FVC z-score, mean (sd)	-0.27 (0.97)
	Pre-bronchodilator FEV_1/FVC z-score, mean (sd)	-0.16 (1.01)
	Low education level†, %	7.7
	Occupational class‡, %	
	managers and professionals (non-manual)	37.2
	technicians and associate professionals	15.3
	other non-manual workers	8.4
	skilled manual workers	14.6
	semi-skilled or unskilled manual workers	11.3
	not occupationally active, unclassifiable or unknown	13.2
	Smoking initiation, %	
	<15 years of age	9.1

	≥15 years of age	52.6
	never smoking	38.3

* Both grandparents were reported to have only studied up to the minimum school-leaving age.

† Less than or equal to the minimum school-leaving age in their country before the start of the ECRHS [28].

‡ Derived from the longest-held job during the follow-up period between ECRHS I and II, using the International Standard Classification of Occupations (ISCO) [29].

Table 2. Main characteristics of the offspring and offspring-reported information on their mothers.

Generation		N° of offspring = 383
Mother (G1)	Smoking before or after offspring's birth, %	
	present	50.7
	absent	25.3
	unknown	24.0
Offspring (G2)	Female, %	52.0
	Age (years), median (range)	28 (18-47)
	Pre-bronchodilator FEV_1 z-score, mean (sd)	-0.38 (0.75)
	Pre-bronchodilator FVC z-score, mean (sd)	-0.23 (0.83)
	Pre-bronchodilator FEV_1/FVC z-score, mean (sd)	-0.26 (0.91)
	Ever smoking, %	30.3
	Education level, %	
	low*	37.9
	high	57.4
	unknown	4.7

* Offspring had only studied up to the minimum school-leaving age.

Table 3. Natural direct effects* on fathers' and offspring's pre-bronchodilator FEV_1 and FVC (model 1), or FEV_1/FVC (model 2) z-scores within the paternal line.

GENERATION		Father (G1)			Offspring (G2)		
		ΔFEV_1 (95%CI)	ΔFVC (95%CI)	$\Delta FEV_1/FVC$ (95%CI)	ΔFEV_1 (95%CI)	ΔFVC (95%CI)	$\Delta FEV_1/FVC$ (95%CI)
Grandparents (G0)	Grandmother's smoking†						
	when the father was *in utero*	-0.33 (-0.89, 0.23)	-0.05 (-0.54, 0.44)	**-0.57 (-1.09, -0.05)**	-0.03 (-0.35, 0.28)	-0.03 (-0.39, 0.34)	0.01 (-0.09, 0.12)
	before pregnancy or during father's childhood (or unknown smoking period)	-0.27 (-0.70, 0.17)	-0.11 (-0.35, 0.13)	-0.36 (-0.82, 0.11)	0.05 (-0.05, 0.14)	0.05 (-0.08, 0.18)	0.02 (-0.21, 0.25)
Father (G1)	Smoking initiation (*vs* never smoking)						
	<15 years of age	-0.22 (-0.78, 0.34)	-0.05 (-0.64, 0.53)	**-0.36 (-0.68, -0.04)**	**-0.36 (-0.63, -0.10)**	**-0.50 (-0.80, -0.20)**	0.30 (-0.01, 0.61)
	≥15 years of age	**-0.27 (-0.51, -0.02)**	-0.11 (-0.32, 0.11)	**-0.20 (-0.37, -0.04)**	-0.01 (-0.18, 0.16)	-0.04 (-0.23, 0.16)	0.03 (-0.13, 0.20)
	Pre-bronchodilator FEV_1 z-score (1-unit increase)	-	-	-	**0.18 (0.05, 0.31)**	-	-
	Pre-bronchodilator FVC z-score (1-unit increase)	-	-	-	-	**0.17 (0.07, 0.27)**	-
	Pre-bronchodilator FEV_1/FVC z-score (1-unit increase)	-	-	-	-	-	**0.21 (0.07, 0.34)**

* Difference (Δ) in offspring's expected z-score for the change in exposure status, keeping fathers' z-score at its expected value when the exposure is absent. Models 1 and 2 also include the potential confounders and adjusting variables of the mediators (grandparents' education level, and fathers' age, education level and occupational class) and of the outcomes (fathers' education level and occupational class, mother's smoking before or after offspring's birth, and offspring's age, sex, education level and smoking).

† *Vs* not smoking before father's birth nor during father's childhood.

The estimates in bold are statistically significant (p-value <0.05).

Table 4. Natural indirect effects* on offspring's pre-bronchodilator FEV_1 and FVC (model 1), or FEV_1/FVC (model 2) z-scores within the paternal line.

GENERATION		Offspring (G2)		
		ΔFEV_1 (95%CI)	ΔFVC (95%CI)	$\Delta FEV_1/FVC$ (95%CI)
Grandparents (G0)	Grandmother's smoking†			
	when the father was *in utero*	-0.06 (-0.17, 0.05)	-0.01 (-0.09, 0.08)	**-0.12 (-0.21, -0.03)**
	before pregnancy or during father's childhood (or unknown smoking period)	-0.05 (-0.13, 0.03)	-0.02 (-0.06, 0.02)	-0.07 (-0.17, 0.02)
Father (G1)	Smoking initiation (*vs* never smoking)			
	<15 years of age	-0.04 (-0.14, 0.06)	-0.01 (-0.10, 0.09)	-0.07 (-0.18, 0.02)
	≥15 years of age	-0.05 (-0.10, 0.004)	-0.02 (-0.06, 0.02)	-0.04 (-0.09, 0.01)

* Difference (Δ) in offspring's expected z-score when the exposure is present, but fathers' z-score changes from its expected value when the exposure is absent to its expected value when the exposure is present. Models 1 and 2 also include the potential confounders and adjusting variables of the mediators (grandparents' education level, and fathers' age, education level and occupational class) and of the outcomes (fathers' education level and occupational class, mother's smoking before or after offspring's birth, and offspring's age, sex, education level and smoking).

† *Vs* not smoking before father's birth nor during father's childhood.

The estimates in bold are statistically significant (p-value <0.05).

FIGURE LEGENDS

Figure 1. Selection of the study subjects (fathers and their offspring).

Figure 2. Mediation model for FEV_1 and FVC within the paternal line (model 1).

The green boxes represent the exposures of interest, the yellow boxes the mediators and the blue boxes the outcomes. The dotted boxes represent the set of potential confounders and adjusting variables of the mediators (X_1: grandparents' education level, and fathers' age, education level and occupational class) and of the outcomes (X_2: fathers' education level and occupational class, mother's smoking before or after offspring's birth, and offspring's age, sex, education level and smoking). The two ellipses represent the level 2 unit (father; the arrows indicate the random intercept terms at level 2) and the cluster variable (country; no arrow indicates that cluster-robust standard errors were computed in order to take the correlation among fathers within countries into account).

Figure 3. Mediation model for FEV_1/FVC within the paternal line (model 2).

The green boxes represent the exposures of interest, the yellow box the mediator and the blue box the outcome. The dotted boxes represent the set of potential confounders and adjusting variables of the mediator (X_1: grandparents' education level, and fathers' age, education level and occupational class) and of the outcome (X_2: fathers' education level and occupational class, mother's smoking before or after offspring's birth, and offspring's age, sex, education level and smoking). The two ellipses represent the level 2 unit (father; the arrow indicates the random intercept term at level 2) and the cluster variable (country; no arrow indicates that cluster-robust standard errors were computed in order to take the correlation among fathers within countries into account).

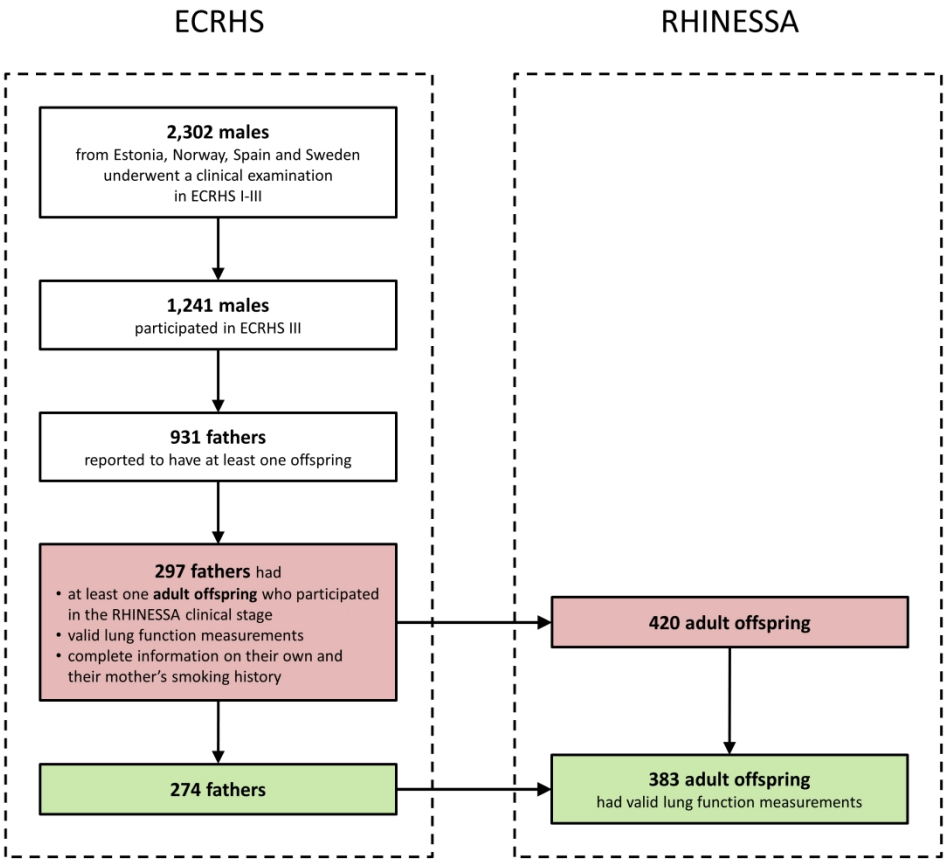

Figure 1. Selection of the study subjects (fathers and their offspring).

820x755mm (96 x 96 DPI)

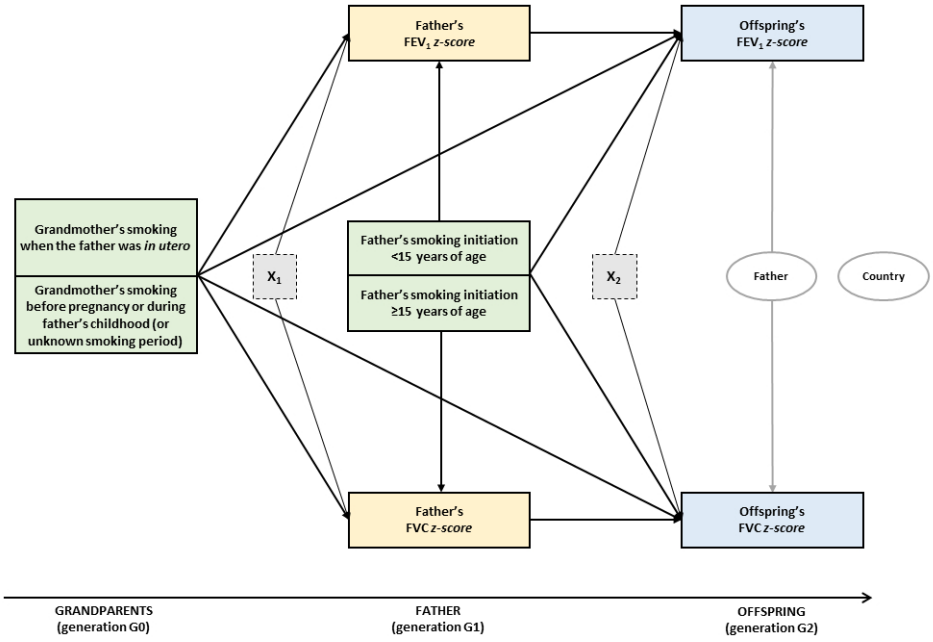

Figure 2. Mediation model for FEV1 and FVC within the paternal line (model 1).

275x189mm (96 x 96 DPI)

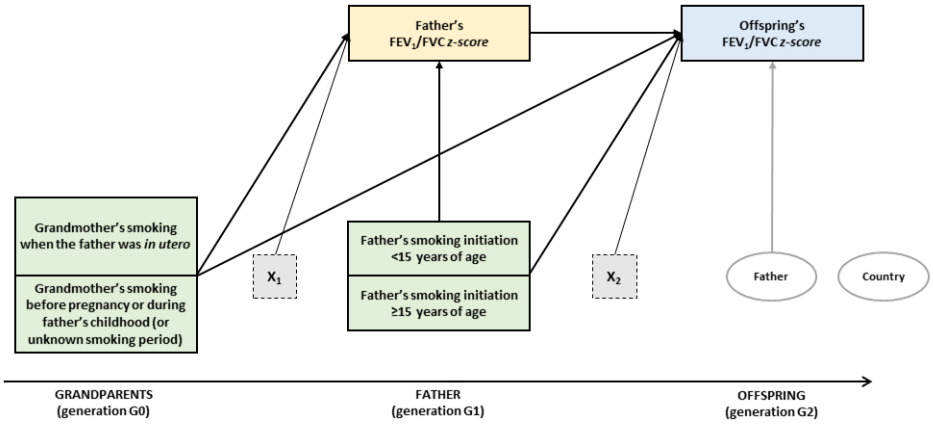

Figure 3. Mediation model for FEV1/FVC within the paternal line (model 2).

275x129mm (96 x 96 DPI)

MINIMAL SUFFICIENT ADJUSTMENT SET

We explored whether the potential confounders included in *model 1* and *model 2* represent the "*minimal sufficient adjustment set*" to estimate the total effect of each exposure on the outcomes (i.e. the smallest group of measured covariates that needs to be included in order to eliminate confounding). We used directed acyclic graphs (DAGs) [1] in DAGitty (*dagitty.net*) (**figure S1** and **figure S2**). DAGs help to minimize the magnitude of the bias in the estimates, to avoid the risk of over-adjustment and to establish whether the statistical models used are the most parsimonious.

The DAG analysis supported the assumption that the minimal sufficient adjustment set contains grandparents' education level, fathers' age, education level and occupational class, mother's smoking before or after offspring's birth, and offspring's age, education level, sex and smoking ("*education_GP*", "*age_F*", "*education_F*", "*occupation_F*", "*smoke_M*", "*age_O*", "*education_O*"', "*sex_O*" and "*smoke_O*" in **figure S1** and **figure S2**).

UNMEASURED CONFOUNDING

We evaluated the impact of unmeasured confounding [2] on the estimate of the natural direct and indirect effects of fathers' and grandmothers' smoking on offspring's lung function, using the Umediation package (*github.com/SharonLutz/Umediation*) in R3.6.1. Umediation makes it possible to simulate unmeasured confounding of the exposure-outcome, exposure-mediator and mediator-outcome relationships in order to investigate how the results would change if up to two unmeasured confounders were included in the mediation models.

We carried out the simulation analyses as follows:

- In *model 1* and *model 2*, we subdivided the paths in order to simulate unmeasured confounding within a single-exposure, single-mediator, single-outcome framework. Then, we added up to two unmeasured normally distributed confounders with mean 0 and variance 0.001 to the models ("U_1" and "U_2" variables). **Figure S3** shows how the data were simulated for "*smoke_GM*" (exposure), "*FEV1_F*" (mediator) and "*FEV1_O*" (outcome).
- As inputs for the simulations, we used the beta regression coefficients obtained from the cluster-robust (cluster variable: centre) linear models, defined as follows:
  - "*FEV1_O*" as the outcome and "*smoke_GM*", "*education_F*", "*FEV1_F*", "*occupation_F*", "*smoke_F*", "*smoke_M*", "*age_O*", "*education_O*", "*sex_O*", "*smoke_O*" as covariates;
  - "*FVC_O*" as the outcome and "*smoke_GM*", "*education_F*", "*FVC_F*", "*occupation_F*", "*smoke_F*", "*smoke_M*", "*age_O*", "*education_O*", "*sex_O*", "*smoke_O*" as covariates;
  - "*FEV1/FVC_O*" as the outcome and "*smoke_GM*", "*education_F*", "*FEV1/FVC_F*", "*occupation_F*", "*smoke_F*", "*smoke_M*", "*age_O*", "*education_O*", "*sex_O*", "*smoke_O*" as covariates;
  - "*FEV1_F*" as the outcome and "*education_GP*", "*smoke_GM*", "*age_F*", "*education_F*", "*occupation_F*", "*smoke_F*" as covariates;
  - "*FVC_F*" as the outcome and "*education_GP*", "*smoke_GM*", "*age_F*", "*education_F*", "*occupation_F*", "*smoke_F*" as covariates;
  - "*FEV1/FVC_F*" as the outcome and "*education_GP*", "*smoke_GM*", "*age_F*", "*education_F*", "*occupation_F*", "*smoke_F*" as covariates;
  - "*smoke_GM*" as the outcome and no covariates (null model);
  - "*smoke_F*" as the outcome and no covariates (null model).

  The beta regression coefficients were estimated from 800 bootstrap samplings of one offspring per parent (n = n° offspring = n° fathers = 274). This was done to avoid the "*2→2→1*" mediation pattern.
- We carried out the simulations under multiple scenarios for the effects (beta regression coefficients) of the unmeasured confounder "U_1" on "*smoke_GM*" and "*smoke_F*" (exposure; $beta_{U\rightarrow E}$), on "*FEV1_F*", "*FVC_F*" and "*FEV1/FVC_F*" (mediator; $beta_{U\rightarrow M}$), and on "*FEV1_O*", "*FVC_O*" and "*FEV1/FVC_O*" (outcome; $beta_{U\rightarrow O}$), by fixing $beta_{U\rightarrow E} = beta_{U\rightarrow M} = beta_{U\rightarrow O} = 0$, 1, 3, 5, 7 and 9. We repeated the simulations by adding the second unmeasured confounder "U_2" to the models under the same assumptions.
- We selected 1,000 simulation runs and 1,000 Monte Carlo draws for the nonparametric bootstrap in each of the simulation analyses.

REFERENCES

1 Greenland S, Pearl J, Robins JM. Causal diagrams for epidemiologic research. Epidemiology 1999; 10: 37-48.
2 Lutz SM, Thwing A, Schmiege S, *et al*. Examining the role of unmeasured confounding in mediation analysis with genetic and genomic applications. BMC Bioinformatics 2017; 18: 344.

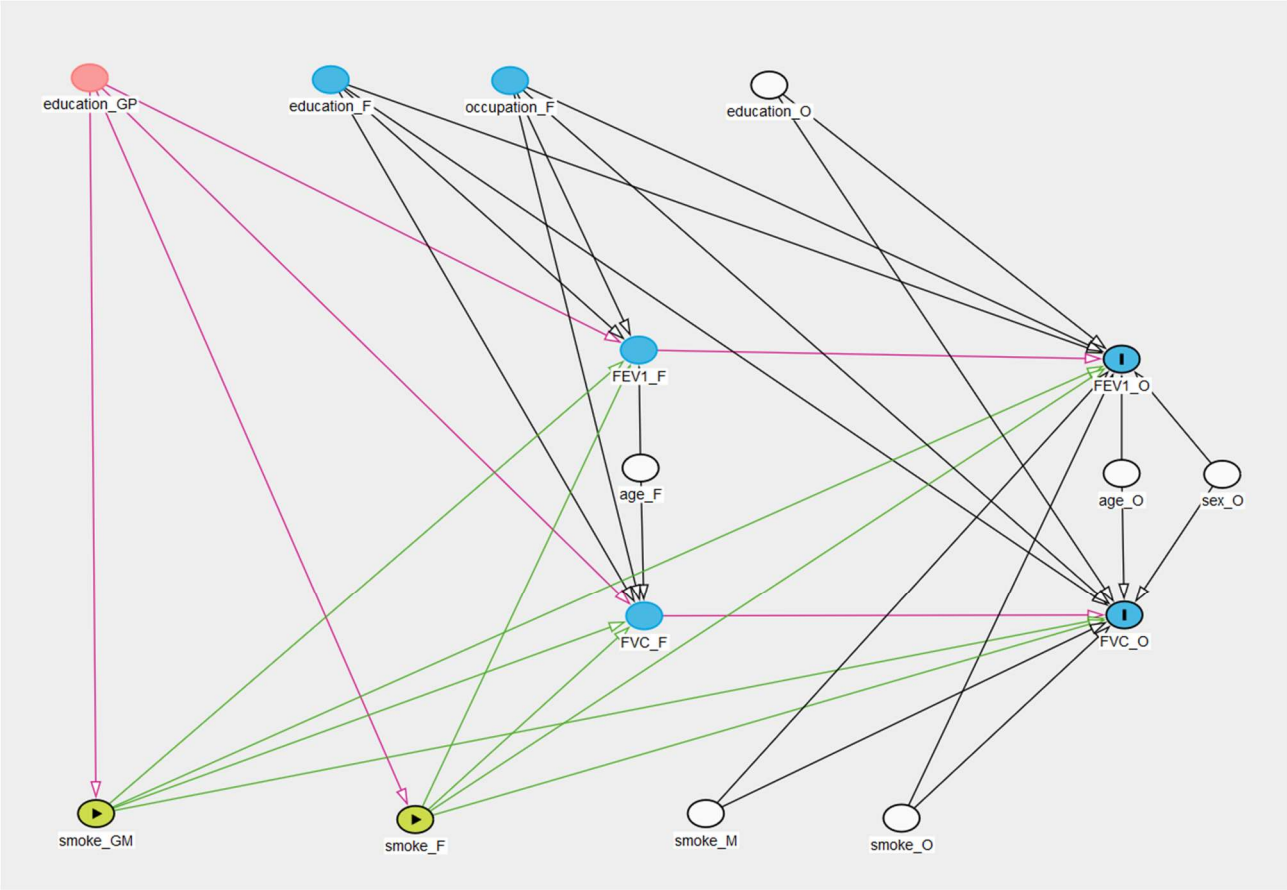

Figure S1. Directed acyclic graph used to check if the potential confounders included in *model 1* represent the "*minimal sufficient adjustment set*". Grandparents (generation G0): "education_GP", grandparents' education level; "smoke_GM", grandmother's smoke. Father/mother (generation G1): "age_F", father's age; "education_F", father's education level; "FEV1_F", father's FEV_1 z-score; "FVC_F", father's FVC z-score; "occupation_F", father's occupational class; "smoke_F", father's smoke; "smoke_M", mother's smoke. Offspring (generation G2): "age_O", offspring's age; "education_O", offspring's education level; "FEV1_O", offspring's FEV_1 z-score; "FVC_O", offspring's FVC z-score; "sex_O", offspring's sex; "smoke_O", offspring's smoke.

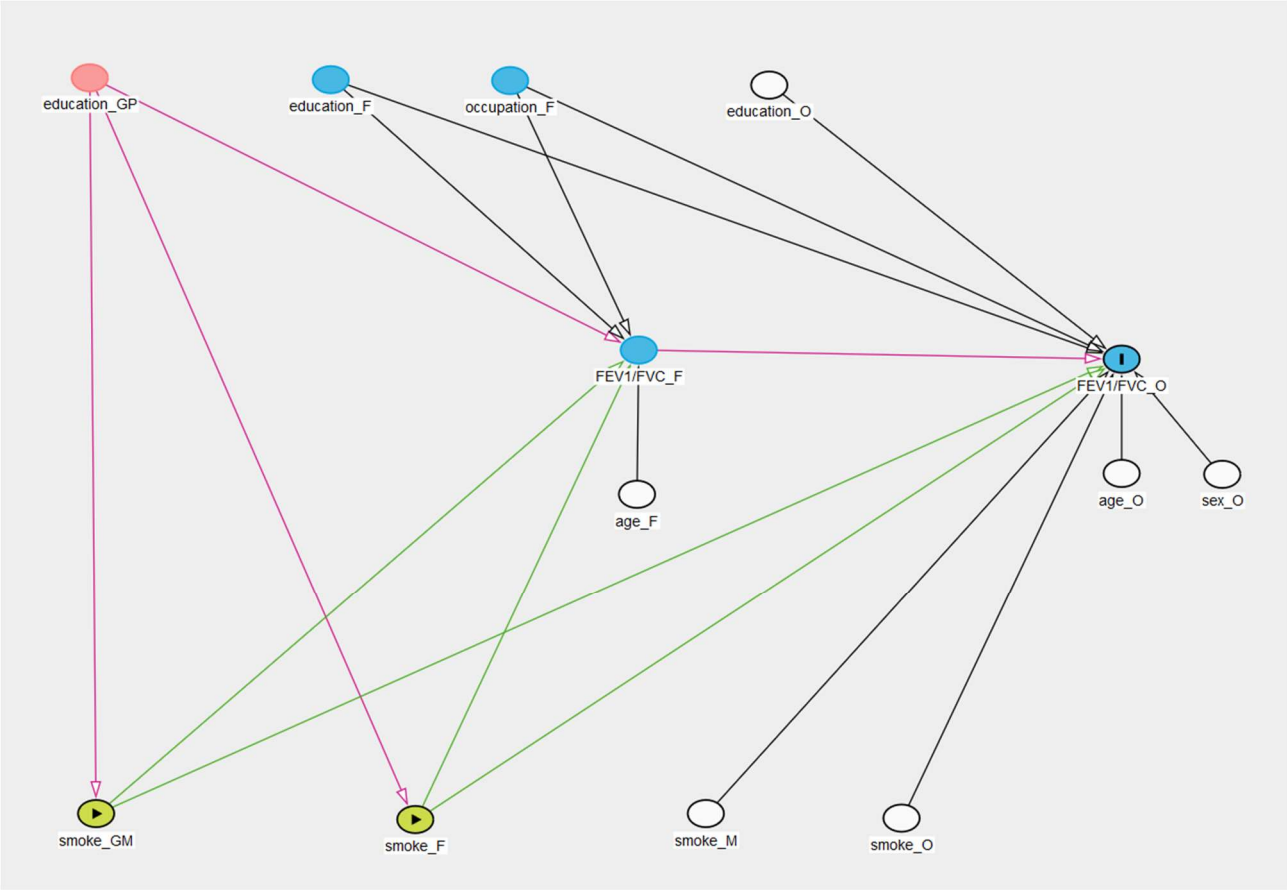

Figure S2. Directed acyclic graph used to check if the potential confounders included in *model 2* represent the "*minimal sufficient adjustment set*". Grandparents (generation G0): "education_GP", grandparents' education level; "smoke_GM", grandmother's smoke. Father/mother (generation G1): "age_F", father's age; "education_F", father's education level; "FEV1/FVC_F", father's FEV_1/FVC z-score; "occupation_F", father's occupational class; "smoke_F", father's smoke; "smoke_M", mother's smoke. Offspring (generation G2): "age_O", offspring's age; "education_O", offspring's education level; "FEV1/FVC_O", offspring's FEV_1/FVC z-score; "sex_O", offspring's sex; "smoke_O", offspring's smoke.

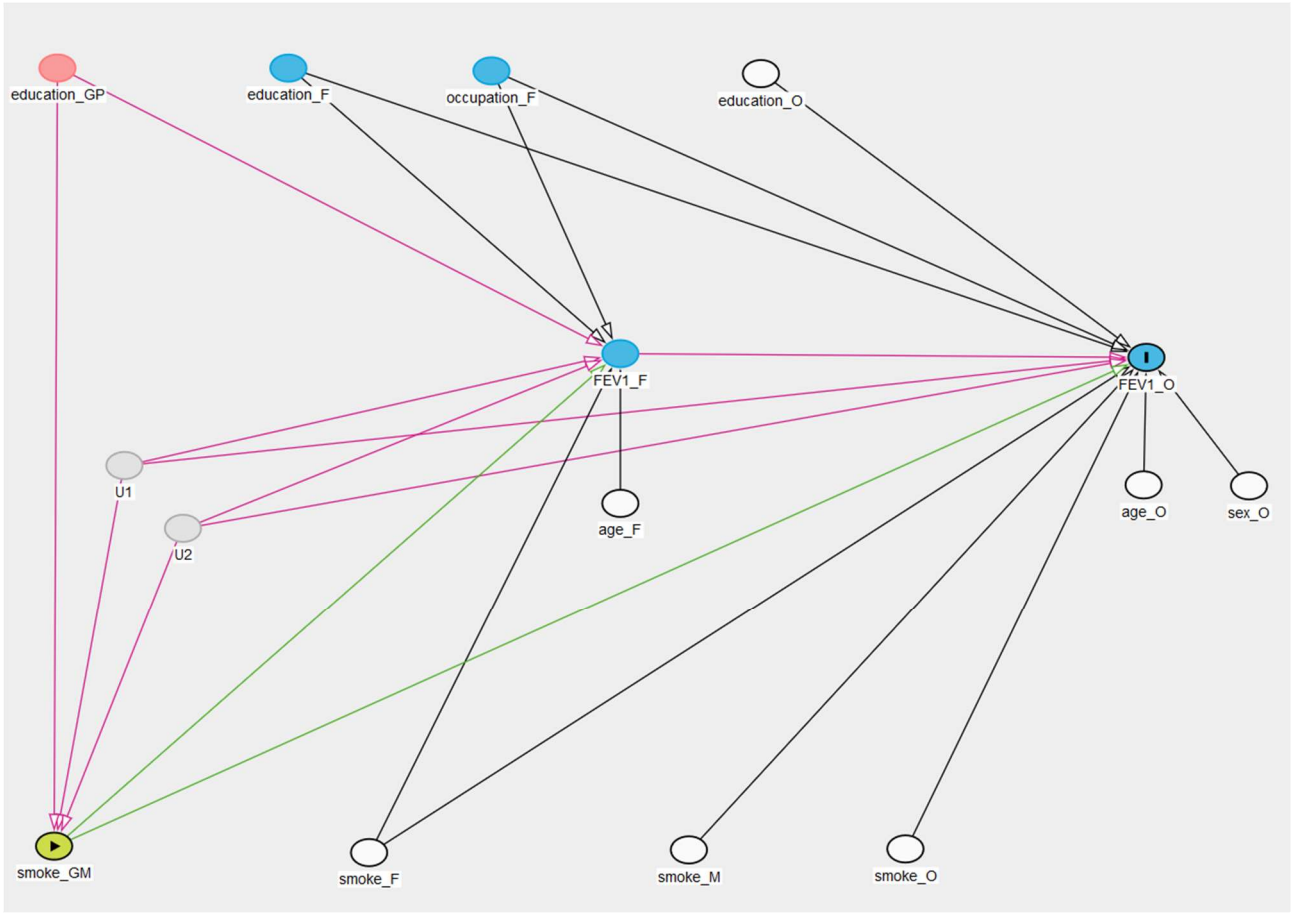

Figure S3. Directed acyclic graph used to simulate the impact of two unmeasured confounders ("U_1" and "U_2") on the direct and indirect effects of grandmothers' smoking in pregnancy on offspring's FEV_1 z-score. Grandparents (generation G0): "education_GP", grandparents' education level; "smoke_GM", grandmother's smoke. Father/mother (generation G1): "age_F", father's age; "education_F", father's education level; "FEV1_F", father's FEV_1 z-score; "occupation_F", father's occupational class; "smoke_F", father's smoke; "smoke_M", mother's smoke. Offspring (generation G2): "age_O", offspring's age; "education_O", offspring's education level; "FEV1_O", offspring's FEV_1 z-score; "sex_O", offspring's sex; "smoke_O", offspring's smoke.

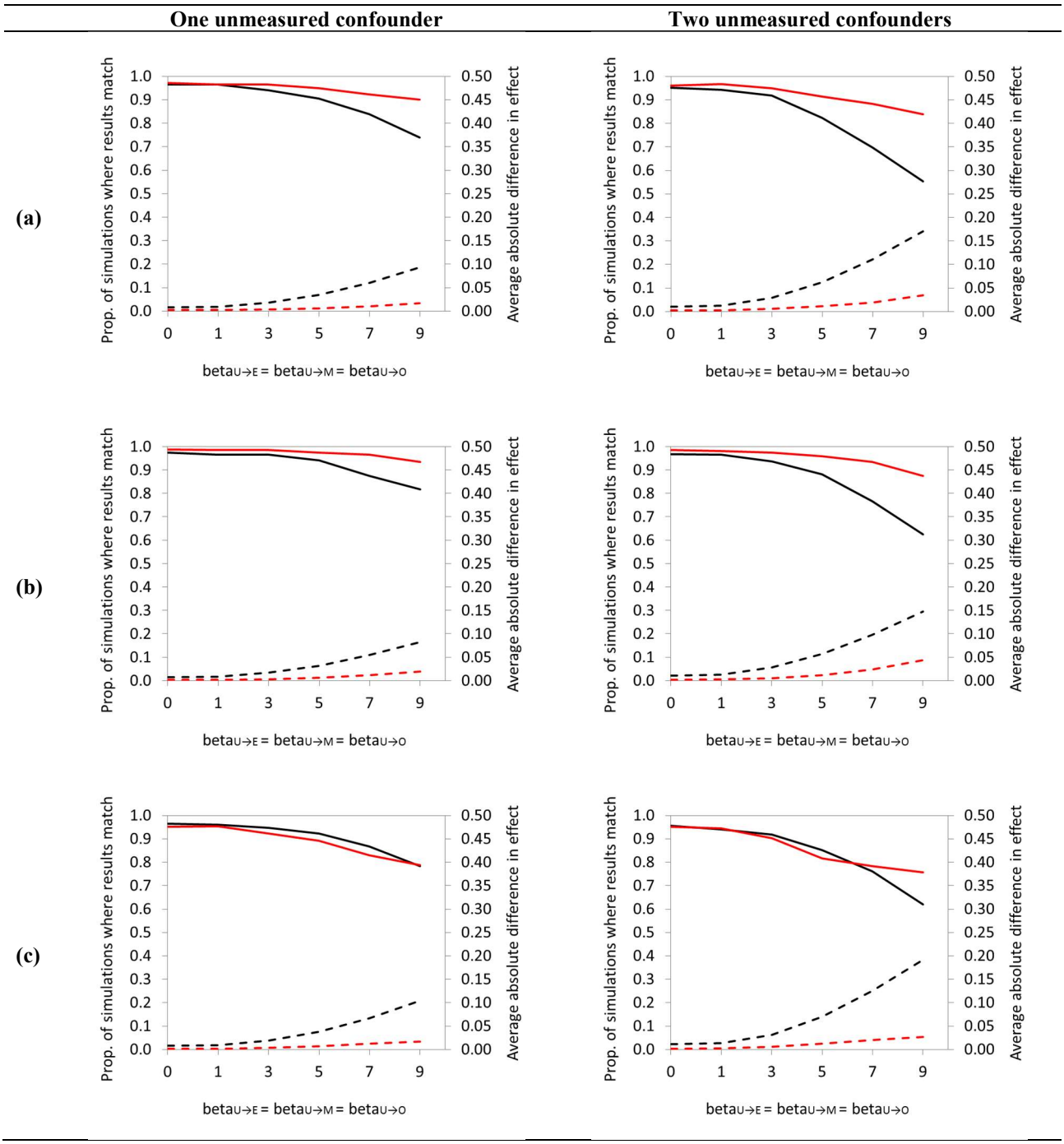

Figure S4. Proportion of Monte Carlo simulations where results match (solid line) and average absolute difference (dashed line) in the average direct (black line) and indirect (red line) effects of fathers' smoking initiation in prepuberty on offspring's lung function (whether one or two unmeasured confounders are included or excluded from the models). Outcomes: (a) FEV_1, (b) FVC and (c) FEV_1/FVC z-scores.

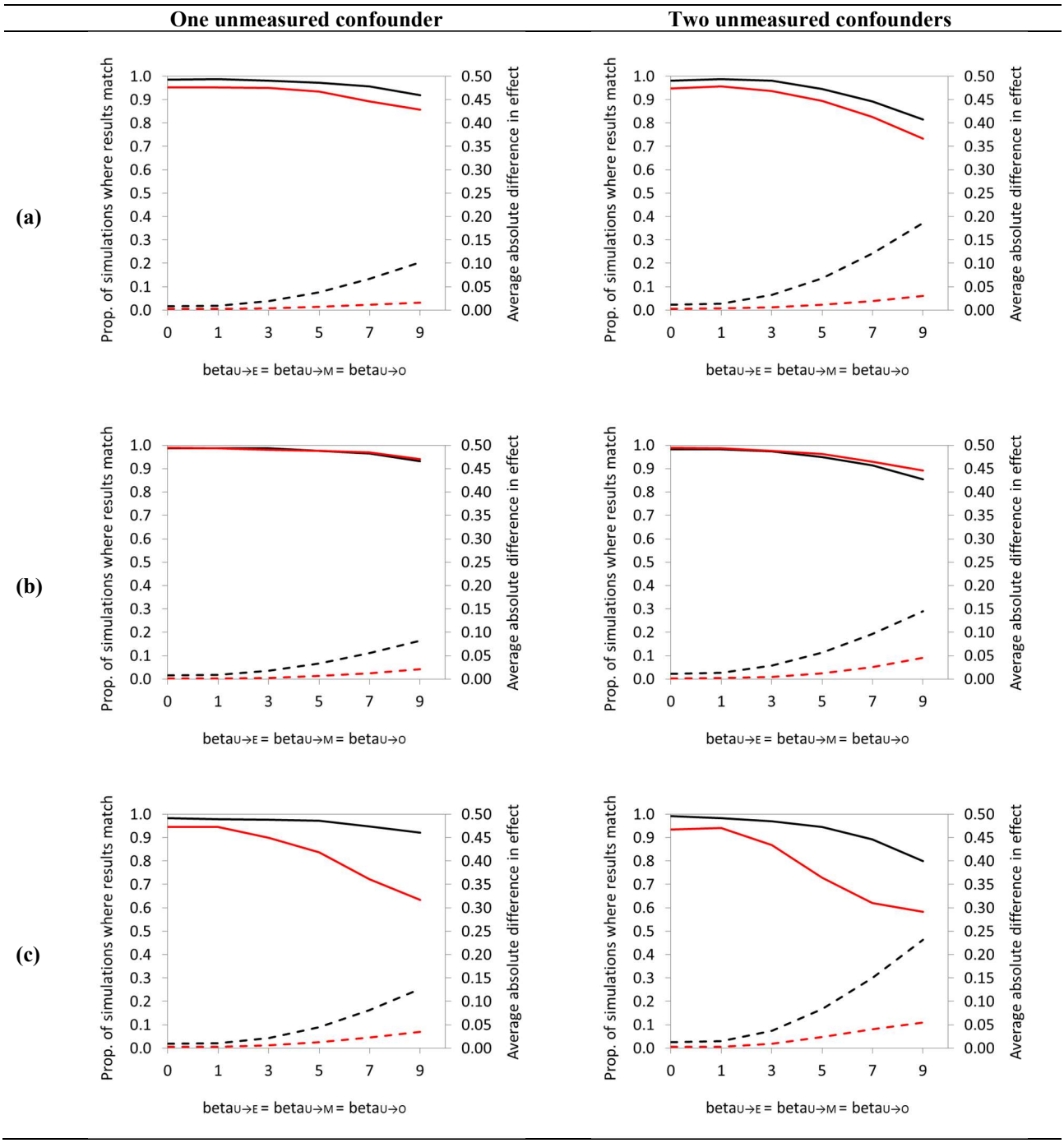

Figure S5. Proportion of Monte Carlo simulations where results match (solid line) and average absolute difference (dashed line) in the average direct (black line) and indirect (red line) effects of grandmothers' smoking in pregnancy on offspring's lung function (whether one or two unmeasured confounders are included or excluded from the models). Outcomes: (a) FEV_1, (b) FVC and (c) FEV_1/FVC z-scores.

SUPPLEMENTARY INFORMATION ON THE EUROPEAN COMMUNITY RESPIRATORY HEALTH SURVEY (ECRHS)

ECRHS I

Co-ordinating Centre (London): P. Burney, S. Chinn, C. Luczynska†, D. Jarvis, E. Lai.

Project Management Group: P. Burney (Project leader-UK), S. Chinn (UK), C. Luczynska† (UK), D. Jarvis (UK), P. Vermeire† (Antwerp), H. Kesteloot (Leuven), J. Bousquet (Montpellier), D. Nowak (Hamburg), J. Prichard† (Dublin), R. de Marco† (Verona), B. Rijcken (Groningen), J.M. Antó (Barcelona), J. Alves (Oporto), G. Boman (Uppsala), N. Nielsen (Copenhagen), P. Paoletti (Pisa).

Financial support: The following grants helped to fund the local studies. **Australia**: Asthma Foundation of Victoria, Allen and Hanbury's; **Belgium**: Belgian Science Policy Office, National Fund for Scientific Research; **Estonia:** Estonian Science Foundation, grant no 1088; **France:** Ministère de la Santé, Glaxo France, Institut Pneumologique d'Aquitaine, Contrat de Plan Etat-Région Languedoc-Rousillon, CNMATS, CNMRT (90MR/10, 91AF/6), Ministre Delegué de la Santé, RNSP; **Germany**: GSF, Bundesminister für Forschung und Technologie; **Italy:** Ministero dell'Università e della Ricerca Scientifica e Tecnologica, CNR, Regione Veneto grant RSF n. 381/05.93; **Norway:** Norwegian Research Council project no. 101422/310; **Spain:** Fondo de Investigación Sanitaria (#91/0016-060-05/E, 92/0319 and #93/0393), Hospital General de Albacete, Hospital General Juan Ramón Jiménez, Dirección Regional de Salud Pública (Consejería de Sanidad del Principado de Asturias), CIRIT (1997 SGR 00079) and Servicio Andaluz de Salud; **Sweden:** The Swedish Medical Research Council, the Swedish Heart Lung Foundation, the Swedish Association against Asthma and Allergy; **Switzerland:** Swiss national Science Foundation grant 4026-28099; **UK:** National Asthma Campaign, British Lung Foundation, Department of Health, South Thames Regional Health Authority.

Coordination: The co-ordination of this work was supported by the European Commission; the authors and participants are grateful to the late C. Baya and M. Hallen for their help during the study, and to K. Vuylsteek and the members of the COMAC for their support.

ECRHS II

Steering Committee: U. Ackermann-Liebrich (University of Basel, Switzerland); N. Kuenzli (University of Basel, and University of Southern California, Los Angeles, USA); J.M. Antó and J. Sunyer (Institut Municipal d' Investigació Médica (IMIM-IMAS), Universitat Pompeu Fabra (UPF), Spain); P. Burney (project leader), S Chinn, D. Jarvis, J. Knox and C. Luczynska (King's College London, UK); I. Cerveri (University of Pavia, Italy); R. de Marco† (University of Verona, Italy); T. Gislason (Iceland University Hospital, Iceland); J. Heinrich and M. Wjst (GSF–Institute of Epidemiology, Germany); C. Janson (Uppsala University, Sweden); B. Leynaert and F. Neukirch (Institut National de la Santé et de la Recherche Medicale (INSERM), France); J. Schouten (University of Groningen, The Netherlands); C. Svanes (University of Bergen, Norway); P. Vermeire† (University of Antwerp, Belgium).

Principal Investigators and senior scientific teams: **Australia: Melbourne** (M. Abramson, E.H. Walters, J. Raven); **Belgium: South Antwerp and Antwerp City** (P. Vermeire, J. Weyler, M. van Sprundel, V. Nelen); **Estonia: Tartu** (R. Jõgi, A. Soon); **France: Paris** (F. Neukirch, B. Leynaert, R. Liard, M. Zureik), **Grenoble** (I. Pin, J. Ferran-Quentin), **Bordeaux** (A. Taytard, C. Raherison), **Montpellier** (J. Bousquet, P.J. Bousquet); **Germany: Erfurt** (J. Heinrich, M. Wjst, C. Frye, I. Meyer); **Iceland: Reykjavik** (T. Gislason, E. Bjornsson, D. Gislason, K.B. Jörundsdóttir); **Italy: Turin** (R. Bono, M. Bugiani, P. Piccioni, E. Caria, A. Carosso, E. Migliore, G. Castiglioni), **Verona** (R. de Marco†, G. Verlato, E. Zanolin, S. Accordini, A. Poli, V. Lo Cascio, M. Ferrari, I. Cazzoletti), **Pavia** (A. Marinoni, S. Villani, M. Ponzio, F. Frigerio, M. Comelli, M. Grassi, I. Cerveri, A. Corsico); **Norway: Bergen** (A. Gulsvik, E. Omenaas, C. Svanes, B. Laerum); **Spain: Albacete** (J. Martinez-Moratalla Rovira, E. Almar, M. Arévalo, C. Boix, G. González, J.M. Ignacio García, J. Solera, J. Damián), **Galdakao** (N. Muñozguren, J. Ramos, I. Urrutia, U. Aguirre), **Barcelona** (J.M. Antó, J. Sunyer, M. Kogevinas, J.P. Zock, X. Basagaña, A. Jaen, F. Burgos, C. Acosta), **Huelva** (J. Maldonado, A. Pereira, J.L. Sanchez), **Oviedo** (F. Payo, I. Huerta, A. de la Vega, L. Palenciano, J. Azofra, A. Cañada); **Sweden**: **Göteborg** (K. Toren, L. Lillienberg, A.C. Olin, B. Balder, A. Pfeifer-Nilsson, R. Sundberg), **Umea** (E. Norrman, M. Soderberg, K.A. Franklin, B. Lundback, B. Forsberg, L. Nystrom), **Uppsala** (C. Janson, G. Boman, D. Norback, G. Wieslander, M. Gunnbjornsdottir); **Switzerland: Basel** (N. Küenzli, B. Dibbert, M. Hazenkamp, M. Brutsche, U. Ackermann-Liebrich); **United Kingdom: Ipswich** (D. Jarvis, R. Hall, D. Seaton), **Norwich** (D. Jarvis, B. Harrison).

Financial Support: **Australia:** National Health and Medical Research Council; **Belgium: Antwerp:** Fund for Scientific Research (G.0402.00), University of Antwerp, Flemish Health Ministry; **Estonia: Tartu:** Estonian Science Foundation grant no 4350; **France: (All)** Programme Hospitalier de Recherche Clinique—Direction de la Recherche Clinique (DRC) de Grenoble 2000 number 2610, Ministry of Health, Ministère de l'Emploi et de la Solidarité, Direction Génerale de la Santé, Centre Hospitalier Universitaire (CHU) de Grenoble; **Bordeaux:** Institut Pneumologique d'Aquitaine; **Grenoble:** Comité des Maladies Respiratoires de l'Isere; **Montpellier:** Aventis (France), Direction Regionale des Affaires Sanitaires et Sociales Languedoc-Roussillon; **Paris:** Union Chimique Belge-Pharma (France), Aventis (France), Glaxo France; **Germany: Erfurt:** GSF—National Research Centre for Environment and Health, Deutsche Forschungsgemeinschaft (FR1526/1-1); **Hamburg:** GSF—National Research Centre for Environment and Health, Deutsche Forschungsgemeinschaft (MA 711/4-1); **Iceland: Reykjavik:** Icelandic Research Council, Icelandic University Hospital Fund; **Italy: Pavia:** GlaxoSmithKline Italy, Italian Ministry of University and Scientific and Technological Research (MURST), Local University Funding for Research 1998 and 1999; **Turin:** Azienda Sanitaria Locale 4 Regione Piemonte (Italy), Azienda Ospedaliera Centro Traumatologico Ospedaliero/Centro Traumatologico Ortopedico—Istituto Clinico Ortopedico Regina Maria Adelaide Regione Piemonte; **Verona:** Ministero dell'Università e della Ricerca Scientifica (MURST), Glaxo Wellcome spa; **Norway: Bergen:** Norwegian Research Council, Norwegian Asthma and Allergy Association, Glaxo Wellcome AS, Norway Research Fund; **Spain: Albacete:** Fondo de Investigaciones Sanitarias (97/0035-01, 99/0034-01, and 99/0034-02), Hospital Universitario de Albacete, Consejeria de Sanidad; **Barcelona:** Sociedad Espanola de Neumología y Cirugía Toracica, Public Health Service (R01 HL62633-01), Fondo de Investigaciones Sanitarias (97/0035-01, 99/0034-01, and 99/0034-02), Consell Interdepartamental de Recerca i Innovació Tecnològica (1999SGR 00241), Instituto de Salud Carlos III, Red de Centros de Epidemiología y Salud Pública (C03/09), Red de Bases moleculares y fisiológicas de las Enfermedades Respiratorias (C03/011), Red de Grupos Infancia y Medio Ambiente (G03/176); **Huelva:** Fondo de Investigaciones Sanitarias (97/0035-01, 99/0034-01, and 99/0034-02); **Galdakao:** Basque Health Department; **Oviedo:** Fondo de Investigaciones Sanitarias (97/0035-02, 97/0035, 99/0034-01, 99/0034-02, 99/0034-04, 99/0034-06, 99/350, and 99/0034-07), European Commission (EU-PEAL PL01237), Generalitat de Catalunya (CIRIT 1999 SGR 00214), Hospital Universitario de Albacete, Sociedad Española de Neumología y Cirugía Torácica (SEPAR R01 HL62633-01), Red de Centros de Epidemiología y Salud Pública (C03/09), Red de Bases moleculares y fisiológicas de las Enfermedades Respiratorias (C03/011), Red de Grupos Infancia y Medio Ambiente (G03/176, 97/0035-01, 99/0034-01, and 99/0034-02); **Sweden: Göteborg, Umea, and Uppsala**: Swedish Heart Lung Foundation, Swedish Foundation for Health Care Sciences and Allergy Research, Swedish Asthma and Allergy Foundation, Swedish Cancer and Allergy Foundation, Swedish Council for Working Life and Social Research (FAS); **Switzerland: Basel:** Swiss National Science Foundation, Swiss Federal Office for Education and Science, Swiss National Accident Insurance Fund; **UK: Ipswich and Norwich**: Asthma UK (formerly known as National Asthma Campaign).

Cordination: The coordination of this work was supported by the European Commission, as part of their Quality of Life programme (QLK4-CT-1999-01237).

ECRHS III

Principal Investigators and senior scientific teams: **Australia: Melbourne** (M. Abramson, G. Benke, S. Dharmage, B. Thompson, S. Kaushik, M. Matheson). **Belgium: South Antwerp & Antwerp City** (J. Weyler, H. Bentouhami, V. Nelen). **Estonia: Tartu** (R. Jõgi, H.Orru). **France: Bordeaux** (C. Raherison, P.O. Girodet), **Grenoble** (I. Pin, V. Siroux, J. Ferran, J.L. Cracowski), **Montpellier** (P. Demoly, A. Bourdin, I. Vachier), **Paris** (B. Leynaert, D. Soussan, D. Courbon, C. Neukirch, L. Alavoine, X. Duval, I. Poirier). **Germany: Erfurt** (J. Heinrich, E. Becker, G. Woelke, O. Manuwald), **Hamburg** (H. Magnussen, D. Nowak, A-M. Kirsten). **Iceland: Reykjavik** (T. Gislason, B. Benediktsdottir, D. Gislason, E.S. Arnardottir, M. Clausen, G. Gudmundsson, L. Gudmundsdottir, H. Palsdottir, K. Olafsdottir, S. Sigmundsdottir, K. Bara-Jörundsdottir). **Italy**: **Pavia** (I. Cerveri, A. Corsico, A. Grosso, F. Albicini, E. Gini, E.M. Di Vincenzo, V. Ronzoni, S. Villani, F. Campanella, M. Gnesi, F. Manzoni, L. Rossi, O. Ferraro), **Turin** (M. Bugiani, R. Bono, P. Piccioni, R. Tassinari, V. Bellisario, G. Trucco), **Verona** (R. de Marco†, S. Accordini, L. Calciano, L. Cazzoletti, M. Ferrari, A.M. Fratta Pasini, F. Locatelli, P. Marchetti, A. Marcon, E. Montoli, G. Nguyen, M. Olivieri, C. Papadopoulou, C. Posenato, G. Pesce, P. Vallerio, G. Verlato, E. Zanolin). **Norway: Bergen** (C. Svanes, E. Omenaas, A. Johannessen, T. Skorge, F. Gomez Real). **Spain: Albacete** (J. Martinez-Moratalla Rovira, E. Almar, A. Mateos, S. García, A. Núñez, P. López, R. Sánchez, E. Mancebo), **Barcelona** (J.M. Antó, J.P. Zock, J. Garcia-Aymerich, M. Kogevinas, X. Basagaña, A.E. Carsin, F. Burgos, C. Sanjuas, S. Guerra, B. Jacquemin, P. Davdand), **Galdakao** (N. Muñozguren, I. Urrutia, U. Aguirre, S. Pascual), **Huelva** (J. Antonio Maldonado, A. Pereira, J. Luis Sánchez, L. Palacios), **Oviedo** (F. Payo, I. Huerta, N. Sánchez, M. Fernández, B. Robles). **Sweden: Göteborg** (K. Torén, M. Holm, J-L. Kim, A-C. Olin, A. Dahlman-Höglund), **Umea** (B. Forsberg, L. Braback, L. Modig, B. Järvholm, H. Bertilsson, K.A. Franklin, C. Wahlgreen), **Uppsala** (B. Andersson, D. Norback, U. Spetz Nystrom, G. Wieslander,

G.M. Bodinaa Lund, K. Nisser). **Switzerland: Basel** (N.M. Probst-Hensch, N. Künzli, D. Stolz, C. Schindler, T. Rochat, J.M. Gaspoz, E. Zemp Stutz, M. Adam, C. Autenrieth, I. Curjuric, J. Dratva, A. Di Pasquale, R. Ducret-Stich, E. Fischer, L. Grize, A. Hensel, D. Keidel, A. Kumar, M. Imboden, N. Maire, A. Mehta, H. Phuleria, M. Ragettli, M. Ritter, E. Schaffner, G.A. Thun, A. Ineichen, T. Schikowski, M. Tarantino, M. Tsai). **UK: London** (P. Burney, D. Jarvis, S. Kapur, R. Newson, J. Potts), **Ipswich** (N. Innes), **Norwich** (A. Wilson).

Financial Support: **Australia:** National Health & Medical Research Council; **Belgium: Antwerp South, Antwerp City:** Research Foundation Flanders (FWO) (G.0.410.08.N.10); **Estonia: Tartu:** Estonian Ministry of Education (SF0180060s09); **France:** (**All centres**) Ministère de la Santé, Programme Hospitalier de Recherche Clinique (PHRC) national 2010; **Bordeaux:** INSERM U897 Université Bordeaux segalen; **Grenoble:** Comite Scientifique AGIRadom 2011; **Paris:** Agence Nationale de la Santé, Région Ile de France, domaine d'intérêt majeur (DIM); **Germany: Erfurt:** German Research Foundation (HE 3294/10-1); **Hamburg:** German Research Foundation (MA 711/6-1, NO 262/7-1); **Iceland: Reykjavik:** The Landspitali University Hospital Research Fund, University of Iceland Research Fund, ResMed Foundation, California, USA, Orkuveita Reykjavikur (Geothermal plant), Vegagerðin (The Icelandic Road Administration (ICERA). **Italy: (All centres)** Italian Ministry of Health, Chiesi Farmaceutici SpA; **Verona:** Cariverona foundation, Education Ministry (MIUR). **Norway:** Norwegian Research council (214123), Western Norway Regional Health Authorities (911631), Bergen Medical Research Foundation. **Spain:** Fondo de Investigación Sanitaria (PS09/02457, PS09/00716, 09/01511, PS09/02185, PS09/03190), Servicio Andaluz de Salud, Sociedad Española de Neumología y Cirurgía Torácica (SEPAR 1001/2010), Fondo de Investigación Sanitaria (PS09/02457); **Barcelona:** Fondo de Investigación Sanitaria (FIS PS09/00716); **Galdakao:** Fondo de Investigación Sanitaria (FIS 09/01511); **Huelva:** Fondo de Investigación Sanitaria (FIS PS09/02185), Servicio Andaluz de Salud; **Oviedo:** Fondo de Investigación Sanitaria (FIS PS09/03190). **Sweden: (All centres)** The Swedish Heart and Lung Foundation, The Swedish Asthma and Allergy Association, The Swedish Association against Lung and Heart Disease, Swedish Research Council for health, working life and welfare (FORTE); **Göteborg:** Swedish Council for Working life and Social Research; **Umea:** Vasterbotten Country Council ALF grant. **Switzerland:** The Swiss National Science Foundation (33CSCO-134276/1, 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 Aargan, 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). **UK:** Medical Research Council (92091). Support also provided by the National Institute for Health Research through the Primary Care Research Network.

Coordination: The coordination was funded through the Medical Research Council (92091).

† deceased

Ethics approval: Ethics approval was obtained by all centres from the appropriate ethics committees: **Antwerp City and Antwerp South:** Adviescommissie Medische Ethiek UZA-UA (CME); **Tartu:** Research Ethics Committee of the University of Tartu, Estland (N° 209T-17); **French centres:** Comite de protection des personnes, Sud V Est (N° 2011-A00013-38); **German centres:** Ethik-Kommission der Bayerischen Landesarztekammer (N° 10015); **Reykjavik:** National Biotecs Committe of Iceland (NBCI) (N° VSNb2011090016/03.11); **Pavia:** Fondazione IRCCS Policlinico 'San Matteo' (N° P-20110024215); **Turin:** Comitato Etico dell'Azienda Sanitaria Locale TO/2 di Torino (N° 569/09/08); **Verona:** Comitato Etico per la Sperimentazione dell'Azienda Ospedaliera Istituti Ospitalieri di Verona (N° 1393); **Bergen:** Universitetet i Bergen, Regional komité for medisinsk og helsefaglig forskningsetikk, Vest-Norge (REK Vest) (N° 2010/759); **Albacete:** Comité de Ética e Investigación de Complejo Hospitalario de Albacete (N° 04/09); **Barcelona:** Comité Ético de Investigación Clínica del Instituto Municipal de Asistencia Sanitaria, Barcelona, Spain (N° PS09/00716); **Galdakao:** Comité Éticode Investigación del Hospital de Galdakao, Spain (N° 20101104); **Huelva:** Comisión de Investigación del Hospital Juan Ramón Jiménez de Huelva (N° 20090417); **Oviedo:** Comité Ético de Investigación Clínica Regional, Hospital Universitario Central de Asturias (N° 20110415); **Swedish centres:** Ethics Committee at the Medical Faculty, Uppsala University (N° 1999/313 and 2010/068); **Basel:** Swiss Academy of Medical Sciences and the ethics committee of Basel (N° PV123/00,157/00); **UK centres:** NRES Committee London - Stanmore (REC Reference 11/LO/0965 IRAS number 70769).