Maternal smoking during pregnancy affects adult onset of asthma in offspring: a follow up from birth to age 46 years

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Competing interest statement

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Transparency declaration

The corresponding author affirms that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned have been explained.

Dissemination declaration

After accepting of the manuscript, a press release will be delivered to media and patient organizations.

Running title (50 characters)

Maternal smoking and asthma onset in adult offspring

Author contribution:

All authors participated on the planning and conception of the study and the analytical strategy, BX, ST-S and AL wrote the manuscript. STS, BX, AL, KD, JL and JP have performed data management and analyses. All authors have performed critical review of the manuscript.

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Abstract

Rationale Environmental tobacco smoke exposure (ETS) increases asthma risk in children. There is limited knowledge of prenatal ETS for adult onset asthma.

Objectives To determine the association between prenatal ETS and adult onset asthma.

Measurements and Main Results Questionnaire and clinical data of 5200 people, free of physician-diagnosed asthma by the age 31 years, of Northern Finland Birth Cohort 1966 Study was used. The association of maternal smoking during the last three months of pregnancy with onset of physician-diagnosed asthma and with lung function in adult offspring were studied by adjusted multivariate regression analyses. The cumulative incidence of physician-diagnosed asthma between the ages of 31 and 46 years was 5.1% among men and 8.8% among women. Gestational smoke exposure was associated with adult onset asthma among the offspring (adjusted odds ratio 1.54, 95% confidence interval 1.04 to 2.29), namely among offspring who reported either past non-diagnosed asthma (odds ratio 9.63, 95% confidence interval 2.28 to 40.67), or past cough with wheeze (3.21, 95% CI 1.71 to 6.05). Significant association was detected between gestational smoke exposure and offspring´s FEV1/FVC ratio at 31 years of age. In offspring with the haplotype rs11702779-AA of *RUNX1* gestational smoke exposure was associated with adult onset asthma (5.53, 95% CI 2.11 to 14.52, adjusted p for interaction 0.10).

Conclusions Maternal smoking during pregnancy is associated with the cumulative incidence of asthma in offspring between 31-46 years. The association was accentuated in offspring who reported at age 31 as having past respiratory problemsand/or, who had haplotype rs11702779-AA. Also a reduction in FEV1/FVC ratio was observed at age 31 years in offspring with gestational smoke exposure. These results could reflect early vulnerability of offspring´s airways to ETS and its putative long-term effects.

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Key Words: asthma, epidemiology, maternal, offspring, smoking

Abbreviations:

ETS	environmental tobacco smoke
FEV1	forced expiratory volume during the first second
FVC	forced vital capacity
GSTM1	Glutathione S-Transferase Mu 1
GSDMB	Gasdermin B
GSDML	Gasdermin-like isoform GSDML1
GSTP1	Glutathione S-Transferase Pi 1
GSTT1	Glutathione S-Transferase Theta 1
LSM14A	MRNA Processing Body Assembly Factor; Family With Sequence Similarity 61, Member A
MAF	minor allele frequency
ORMDL3	ORM1-like protein 3
PTPRT	Protein Tyrosine Phosphatase Receptor Type T
RUNX1	Runt Related Transcription Factor 1

Summary sentence / Take-Home Message

Maternal smoking during pregnancy is associated with cumulative incidence of asthma in offspring between 31-46 years. The association was accentuated in offspring who reported at age 31 as having past respiratory problems and/or, who had haplotype rs11702779-AA.

Plain Language Summary

Childhood asthma risk is increased by gestational exposure to maternal smoking. Few studies have still addressed the effects of maternal smoking habits during pregnancy on adult asthma onset. We found that maternal smoking during pregnancy associates with new asthma diagnosis in adult offspring between 31-46 years. The association was accentuated in offspring who reported at age 31 as having past respiratory problems . The asthma risk due to gestational tobacco smoke exposure was increased in offspring who had a mutation in the RUNX1-gene. Risk for adult-onset asthma of the offspring could be reduced and airway health could be improved by encouraging women planning for pregnancy and pregnant mothers, to permanently cease smoking.

Introduction

Smoking during pregnancy is common, with an estimated prevalence of up to 38 % in Europe (REF National, regional, and global prevalence of smoking during pregnancy in the general population: a systematic review and meta-analysis) (1), and is the single largest modifiable risk for all pregnancy-related morbidity and mortality (2-6). Studies have shown that gestational ETS exposure increases the risk for wheezing in childhood or asthma among offspring during early childhood (7,8), in preschool-age children (9) in adolescents (10,11), and in adults (12,13). Young maternal age at delivery and passive smoking in utero or during childhood have been documented to increase risk for adult-onset asthma (14,15).

Genetic (16-21) and genomic data (22) have identified susceptibility genes affecting asthma risk due to tobacco smoke. These single nucleotide polymorphisms are found in loci of genes such as genes encoding glutathione S-transferase (GSTM1, GSTT1, GSTP1), Gasdermin (GSDMB, GSDML), ORMDL3, Sm protein family (LSM14A), Protein Tyrosine Phosphatase, Receptor (PTPRT), and Runt Related Transcription Factor 1 (RUNX1).

There is still limited knowledge whether gestational smoke exposure is associated with cumulative incidence of asthma or decreased lung function in adult offspring. The aim was to study the association between gestational smoke exposure and incidence of asthma in adult offspring in the Northern Finland Birth Cohort with a follow up of 46 years. We asked smoking habits of pregnant mothers and investigated offspring´s cumulative incidence of adult-onset asthma, and lung function. The hypothesis was that gestational smoke exposure is associated with cumulative incidence of asthma at 46 years of age, and this risk is increased in those having genetic predisposition and/or in those reporting past respiratory problems.

Methods

The study population was based on the Northern Finland Birth Cohort in 1966 consisting of mothers and their children who were born in the northern Finland provinces of Oulu and Lapland in 1966 (23). The total number of births was 12058 in 1966, which covered 96.3% of all births in the area (Please see the Online Supplement). The birth cohort data regarding prenatal and perinatal environment were collected via questionnaires and clinical examinations during mothers' clinical visit during pregnancy. Follow-ups were conducted at the ages 31 years (24), and 46 years. At the age of 46 in 2012-2013, 10300 subjects were alive and traced (85.4%) and were given the opportunity to respond to two questionnaires (25,26). Of these subjects, 7148 (69 %) completed data collection, which accounted 59 % of the original birth cohort population. The study was approved by the Ethics Committee of the Finnish Institute of Occupational Health and by the Ethics Committee of the Northern Ostrobothnia Hospital District. At all stages of the study, the subjects gave written informed consent according to the Declaration of Helsinki.

Data on self-reported physician-diagnosed asthma and self-reported asthma at 46 years (46y) was collected with questionnaires in 2012-2013. For definition of incident asthma between 31-46 years, we used the following question of asthma (own opinion and physician-diagnosed), asked both at 31 years and at 46 years of age : Have you ever had the following symptoms and/or diseases that are associated with airways? Q1: Asthma; own opinion (Never, Yes during the past 12 months, Yes but only previously);Q2: Asthma; doctor diagnosed or treated (No, Yes):

(I) no physician diagnosed asthma at 31 years of age when responding at 31y Q2:"No" or (Q2:"Missing" and Q1:"No").

(II) physician diagnosed asthma at 46 years of age when responding at 46y Q2:"Yes" and (Q1:"Yes, current" or "Yes, only previous" or "Missing").

(III) no physician diagnosed asthma at 46 years of age when responding at 46y Q2:"No" or (Q2:"Missing" and Q1:"No").

The inclusion criterion was that all subjects fulfilled (I) no physician diagnosed asthma at 31 years of age. The incident physician-diagnosed asthma was the outcome measurement and it was defined by either "Yes" (II) physician diagnosed asthma at 46 years of age, or "No" (III) no physician diagnosed asthma at 46 years of age.

A total of 5961 subjects answered the questions at both 31 years and 46 years of age. Of these subjects, 5491 met the inclusion criteria. Final analyses were limited to 5200 subjects with complete information on both mother's smoking status during the last three months of pregnancy and information on asthma at both 31 and 46 years.

Subjects who reported at 46y as having self-reported asthma not diagnosed by a doctor 'during the past 12 months' were labelled 'beginning asthma'. Subjects who reported at 46y as having self-reported asthma not diagnosed by a doctor 'but only previously' were labelled as 'past asthma'. Similarly, subjects who reported at 46y as having wheeze 'during the past 12 months' and were labelled 'current wheeze' and subject who reported at 46y as having had wheeze 'only previously' were labelled as 'past wheeze'. Those who reported current/past wheeze/asthma without a physician diagnosed asthma (both shortened as "respiratory problems") at 31 years of age were included, for evaluation of possible interactions.

Information on maternal smoking during pregnancy and on covariates

Information about maternal smoking during pregnancy was collected via a questionnaire sent to mothers between the 6^{th}-7^{th} month of pregnancy. Mothers were regarded as smokers during pregnancy if they reported to smoke at least 1 cigarette/pipe per day during the last three months of pregnancy. Mothers were considered

as smokers before pregnancy if they reported to smoke at least 1 cigarette/pipe per day during the 12 months before the pregnancy. Change in smoking status during pregnancy was based on the responses (no; gave up; cut down; no change; increased), the two latter responses were pooled due to the small number. We excluded offspring whose mothers had illogically responded or had not responded to these questions during their pregnancy.. Information on covariates was collected during the mothers' clinical visits during pregnancy and delivery, including newborn´s Apgar score (= health index) at the 1st minute, ponderal index = [birth weight (g) x 100/crown-heel length (cm)^3], parity (= the number of times a female has carried pregnancies to a viable gestational age), maternal education and parental asthma. The subjects' own information on education and smoking status at 31 years was collected via postal questionnaires during the 31-year follow-up in 1997.

Spirometry data at 31 years of age

Of the 5200 offspring included in the analyses, we had spirometry data from 3777 offspring at 31 years of age. Spirometry and the variables used have previously been described (27). The variables used were FEV1= Forced expiratory volume (Litres) during the first second, FVC= forced vital capacity, FEV1/FVC= the ratio between FEV1 and FVC.

Genetic data at 31 years of age

We used a set of SNP previously identified in literature having a putative risk of asthma together with tobacco smoke exposure: GSTM1, GSTT1, GSTP1, ADRB2, IL3, GSDMB, GSDML-ORMDL3, GSDMB, LSM14A, PTPRT, RUNX1 (Table S4). Blood samples of the subjects in this cohort were collected for genotyping at age 31 (i.e. in 1997) and genetic data was available for 5,402 individuals. Genotyping was completed using Illumina HumanCNV370DUO Analysis BeadChip and the Beadstudio 3.1 algorithm as previously described (28). The data of the set of SNPs was obtained from GWAS data.

Statistical analyses

Differences in distribution of selected variables was assessed in relation to maternal smoking during pregnancy. Associations of maternal smoking variables such as maternal smoking before pregnancy, during the last three months of pregnancy, amount of smoking before pregnancy and smoking habit changes during pregnancy on development of new asthma between the ages of 31-46 years were examined with Pearson's $\chi 2$ test. Because the four maternal smoking variables highly correlated, only maternal smoking during the last three months of pregnancy was examined in multivariate analyses. This was done using a binary logistic regression analysis adjusted for selected covariates: mother´s educational level, parental asthma, offspring´s educational level and smoking (at 31 years of age), ponderal index (in tertile), Apgar score at the 1st minute and parity. Binary logistic regression was also used to test effect modifiers of the association between maternal smoking and adult onset asthma, and the associations between SNPs and adult onset asthma adjusted for the same selected covariates as previously mentioned. Associations between maternal smoking and lung function values (FEV1and FEV1/FVC ratio) were studied in linear regression models adjusted for the same selected covariates as previously mentioned. Deviation from the Hardy-Weinberg equilibrium was evaluated by the Linkage disequilibrium (LD) calculator (https://www.ensembl.org/Homo_sapiens/Tools/LD) and $r^2 \geq 0.7$ was considered as a LD between SNPs. The biomaRt package in Bioconductor (29) was used for retrieval of all annotation data from Ensemble gene and SNP database for these input human genomic variants. The data for all attributes (e.g. gene symbols, chromosomal coordinates, rs id, allele etc.) was filtered out based on the scope of study. Finally, additional SNP annotation of these human genomic variants was carried by ANNOVAR (30). All annotation database files (e.g. hg19 gnomad_genome and snp138) were downloaded by using the command line and information about minor allele frequency for all the variants was retrieved (Table S4). Rare variants (MAF for Finnish < 0.05) and those variant that were not available in the GWAS data were excluded from analyses. All other analyses were done using SPSS Base 18 Statistical Software Package (SPSS, Chicago, IL, USA).

Results

The proportion of mothers who smoked during the last three months of pregnancy was 8.6% (Table 1). We observed 372 new physician-diagnosed asthma cases between the ages of 31-46 years among 5200 subjects who were free of an asthma diagnosis at the 31 year follow-up (Table 1, S1). The cumulative incidence for new asthma diagnosis was 7.2%, 8.8% and 5.1%, in all, in females and in males, respectively. All maternal smoking variables, associated statistically significantly with asthma onset between the ages of 31-46 years (Table 1, S1). Mothers who either smoked 12 months before pregnancy or smoked during the last three months of pregnancy increased the risk for offspring´s asthma in adulthood (Table S1). The cumulative incidence of asthma was statistically insignificantly higher if mothers did not change smoking habits or increased amount smoked during the pregnancy, as compared to mothers who gave up smoking during pregnancy (Table S1).

Maternal smoking variables correlated highly ($p=0.01$, $r>0.63$, by Spearman rank correlation test). Thus maternal smoking during the last three months of pregnancy was selected for further multivariate analyses. A higher risk for asthma development between the ages of 31-46 years was observed among offspring if mothers smoked at least one cigarette per day during the last three months of pregnancy : the adjusted OR (CI95%) was 1.54 (1.04-2.29) (Table 1).

The cumulative incidence of asthma onset between 31-46 years was significantly higher in female offspring with gestational maternal smoking exposure during the last three months of pregnancy, compared to female offspring without gestational smoke exposure during the last three months of pregnancy (Table 1, S1).A higher risk of asthma development between the ages of 31-46 years was observed among female offspring if mothers smoked at least one cigarette per day during the last three months of pregnancy Table 1, S1). The risk was independent of the covariates adjusted in the multivariate model (Table 1). There was no interaction

between gender and maternal smoking status during the last three months of pregnancy, on cumulative incidence of asthma between the ages of 31 and 46 years (adjusted p=0.45, Table 1).

Gestational smoke exposure and asthma onset of offspring with past respiratory problems

Gestational maternal smoking exposure during the last three months of pregnancy was more strongly associated with offspring´s adult onset physician diagnosed asthma in the offspring subgroup who reported current or past wheeze at baseline at age 31, as compared to offspring with no history of wheezing (Table 1, S3). This difference reached statistical significance for past wheeze (adjusted p value for interaction 0.075, Table 1), but not for current wheeze (adjusted p value for interaction 0.43, Table 1). Similarly, gestational maternal smoking exposure during the last three months of pregnancy was more strongly associated with offspring´s adult onset physician-diagnosed asthma in the offspring subgroup, who reported self-reported non-diagnosed current or past asthma at age 31, as compared to offspring with no history of asthma (Table 1, S2). This difference reached statistical significance for past non-diagnosed asthma (adjusted p value for interaction was 0.072, Table 1), but not for current non-diagnosed asthma at age 31 (adjusted p value for interaction was 0.33, Table 1).

Gestational smoke exposure and offspring lung function at 31 year of age

Of the 5200 offspring included in the analyses, we had spirometry data of 3775 offspring at 31 years of age. Gestational maternal smoking exposure during the last three months of pregnancy was associated significantly with reduced offspring´s FEV1/FVC ratio at 31 years of age (adjusted beta coefficient -0.056, t= -3.24, 95% CI -0.020 to -0.005, p=0.001, Table 2). When performing multivariate analysis by linear regression model, offspring´s regular smoking at age 31 years was also associated statistically significantly with current FEV1/FVC ratio (beta coefficient -0.075, t= -4.29, 95% CI -0.017 to -0.006, p<0.001), whereas other potential

confounding factors did not have a significant association. Gestational maternal smoking exposure during the last three months of pregnancy was not associated significantly with the offspring´s FEV1value at 31 years of age (Table 2). Those in the lowest quintile of FEV1 value at 31 years of age had an increased cumulative incidence of asthma between the ages of 31 and 46 years (OR=1.59 95%CI 1.21-2.10, p=0.001, adjusted by offspring's gender, height and weight), when comparing to the highest quintile. When adjusted by maternal smoking, maternal education level, ponderal index, Apgar score at the 1st minute, parity, parental asthma, offspring´s education and smoking at 31 years the result remained similar (adjusted OR=1.48 95%CI 1.10-2.0, p=0.011). Those having the lowest quintile of FEV1/FVC ratio value at 31 years of age associated with cumulative incidence of asthma between the ages of 31 and 46 years (OR=1.71 95%CI 1.27-2.30, p<0.001; adjusted OR=1.64 95%CI 1.19-2.26, p=0.003). FEV1/FVC ratio at age 31 was not an effect modifier in the association between gestational maternal smoking exposure during the last three months of pregnancy and offspring´s adult onset physician-diagnosed asthma (adjusted p value for interaction was 0.88, Table 2). Nor was FEV1 value at age 31 an effect modifier in this association (adjusted p value for interaction was 0.99, Table 2).

The association of genetic predisposition and maternal smoking on offspring asthma and/or lung function in adulthood

Six out of twelve SNPs of the following genes, ADRB2, IL3, GSDMB, LSM14A, RUNX1, had minor allele frequency (MAF-Finns) over 5% and data available, and were entered in the analyses (Table S4). None of the haplotypes was enriched with certain living municipality of the offspring (data not shown). The SNPs rs8069176 and rs4795400 of GSDMB gene were in linkage disequilibrium (r^2=0.98, D´ =1.00). The other SNPs were not in linkage disequilibrium. In female offspring, rs1042713-GG of ADRB2 was associated with adult onset asthma (adjusted OR=1.74 95%CI 1.06-2.84, p=0.028). The SNPs of genes IL3, GSDMB, LSM14A and RUNX1 were not associated significantly with adult onset asthma (data not shown). In female

offspring, who carried haplotype AA of RUNX1-gene, maternal smoke exposure was associating statistically significantly with adult onset asthma, when adjusted by gender, maternal education, ponderal index, Apgar score at the 1st minute, parity, parental asthma, offspring´s education and smoking at 31 years (p=0.001, adjusted p for interaction 0.10, Table 3). In female offspring, who carried also certain haplotypes of the genes LSM14A, ADRB2, IL3, and GSDMB maternal smoke exposure was associating statistically significantly with adult onset asthma, yet no significant interaction was detected between haplotype and maternal smoke exposure (adjusted p for interaction > 0.3, Table 3). In male offspring, these phenomena were not detected (Table 3). None of the SNPs associated with offspring´s lung function parameters at the age of 31 years (data not shown).

Discussion

This birth cohort study aimed at evaluating association between gestational smoke exposure and cumulative incidence of doctor-diagnosed asthma in offspring between 31 years and 46 years of age. The subjects with past/current respiratory problems were analysed separately as it was hypothesized that they might interact with maternal smoke exposure on the cumulative incidence of asthma in adult offspring. Maternal smoking during pregnancy is associated with cumulative incidence of asthma in adult offspring between 31-46 years. The association was accentuated in offspring who reported at age 31 as having past respiratory problems and/or, who had haplotype rs11702779-AA. In addition a reduction in FEV1/FVC ratio was observed at age 31 years in the offspring with gestational smoke exposure.

Gestational smoke exposure was also associated with adult onset asthma in offspring who carried haplotype rs11702779-AA of *RUNX1*. This association was slightly more prominent in female offspring. These observed relations were independent of several confounders, which reflects the prenatal and postnatal environment.

RUNX1 is encoding a transcription factor that regulates the differentiation of hematopoietic stem cells into mature blood cells, development of the sensory neurons. SNPs in RUNX are associated with airway responsiveness in asthmatic children and these associations are modified by intrauterine smoke exposure (17). In line with this, we were able to detect interaction between RUNX1 haplotype and maternal smoking exposure in offspring´s adult onset asthma. LSM14A encodes Sm protein family, a family of RNA-binding proteins, which are expressed in every cellular organism and are related to pre-mRNA splicing (31,32). A study showed that the allele G of rs1759092 interact with active tobacco smoking in adult onset asthma (22). In line with this, we detected that offspring who carried rs1759092-AG, and who had maternal smoke exposure beared a higher cumulative incidence of asthma in adulthood compared to other haplotypes. The pathomechanisms behind gestational exposure to maternal smoking and asthma onset at middle age are unclear. Grandmother's smoking when pregnant with the mother increased the risk of persistent asthma in the grandchild independently of the mother's smoking status (19,33,34), suggesting trans-generational epigenetic changes. It thus could be possible that offspring with genetic predisposition would have increased adult asthma risk due to epigenetic re-programming after gestational tobacco smoke exposure.

The relationship between gestational smoke exposure and childhood asthma development is well documented and presents a major health problem for generations to come (3,7-11,35). Only few follow-up studies have examined gene-environmental interactions on adult onset of asthma (36,37). A study, in which parental smoking exposure was assessed retrospectively by a questionnaire to adult offspring, has documented that both intrauterine exposure to maternal smoking during pregnancy and postnatal exposure to environmental tobacco smoke adversely affected adult lung function and increased risk for adult-onset asthma (12). In studies in which parental smoking exposure was also retrospectively assessed by a questionnaire to adult offspring, cumulative and recent environmental tobacco smoke exposure was shown to increase the risk of adult-onset asthma (38). Heavy maternal smoking during childhood was shown to predispose to spirometrically defined

COPD (39). A European population based study showed that early life factors, such as maternal age during delivery and maternal smoking, were significantly associated with FEV1 decline of adult offspring (40). A three-generation study of European population showed that fathers' smoking during early adolescence and grandmothers' and mothers' smoking during pregnancy may independently increase the offspring´s risk for ever asthma (41). An Australian 50-year follow-up study of a childhood asthma population showed a synergistic impact of offspring smoking and asthma on lung function (36), which has been documented also in adult population (42). Maternal smoking seems to synergize with personal smoking by increasing airflow limitation in adults (15,42). A study of US children showed that prenatal and postnatal tobacco smoke exposure was not independently associated with airflow obstruction in school-aged children, however it was associated with airflow obstruction in children with asthma (43).

Controversial information exists whether smoking increases the risk of adult-onset asthma: there are studies that have either failed to show association (44,45), or that have not found an increased risk (46), or that have found association only in females (47). There is evidence that parental smoking behaviour is associated with offspring´s smoking uptake risk (48). Thus both parental smoking and inherited smoking behaviour may have an increased risk of adult-onset asthma among offspring. In our study, adjustment by offspring´s reported smoking status at the age of 31 did not alter or did not have an interaction with the observed association between gestational smoke exposure and cumulative incidence of asthma between 31 years and 46 years of age. This might be due to low number of subjects.

The strengths of our study include population-based design, data collection since pregnancy and a follow up of 46 years. The strength is that a detailed questionnaire on smoking habits was given during pregnancy, minimizing the effect of recall bias. We dropped off mothers who responded illogically to the questionnaire of non-smoking and quitting smoking. We focused our study only on offspring who did not have doctor-diagnosed asthma before age of 31 years. Yet we allowed current or past self-reported respiratory problems

at 31 years, in order to dissect out the offspring subgroup having putatively the highest risk of gestational smoking exposure to adult-onset asthma. This allowed us to demonstrate within 473 new adult asthma onsets that gestational smoke exposure directly predisposes towards development of late-onset asthma in middle-aged offspring. This occurred in offspring reporting past respiratory problems, which is in line to previous observations on active or passive smoking (47-49).

This study aimed at evaluating cumulative incidence of doctor-dignosed asthma between 31 years and 46 years of age. We excluded those having current/past physician diagnosed asthma at 31 years of age. We analyzed separately those who reported no/current/past respiratory problems at 31 years of age, as it was hypothesized that they might interact with maternal smoke exposure on the cumulative incidence of asthma in adult offspring. The self-reported physician-diagnosed incident asthma as an outcome measure probably represents relatively well the lung-function -verified asthmatic population, because a documented reversible obstruction of the lung is required for Finnish asthma drug reimbursement right Physicians´ reports are required to include diagnostic lung function test results in addition to background information and clinical exam´s results. Hence, the subjects reporting self-reported asthma at age 31 years could reflect asthma-like symptoms without diagnostic asthma and/or a beginning asthma. Interestingly, we detected an interaction between past wheeze (before 31 years of age), gestational smoke exposure and cumulative incidence of asthma at 46 years of age as compared with the never wheeze group. Accordingly, the adult asthma risk associated with gestational smoke exposure was also higher in the group who reported of self-reported non-diagnosed past asthma as compared to the group who reported never asthma. This may reflect that, gestational smoke exposure is related to offspring´s early respiratory problems and, these both are associated with adult onset asthma.

After adjustments, we also found significant association between gestational smoke exposure and offspring´s FEV1/FVC ratio at 31 years of age, reflecting that the objective findings were similar to the questionnaire

results. Previous studies have indicated that women may stop, reduce, and sometimes restart smoking during pregnancy which reflects nicotine dependence, and which also makes measurements of maternal smoking difficult in epidemiological studies (2,50).

Our study also has some limitations that need to be considered. These include that no objective smoking markers were measured from pregnant mothers. Shortcomings also include that we cannot separate the direct effects of maternal smoking on the developing child from shared genetic and postnatal lifestyle influences on adult asthma risk, including passive smoking in the childhood house as well as other environmental tobacco exposure. It is likely that women who still smoked three months before delivery, continued to smoke during the offspring's childhood. The extent to which maternal smoking in pregnancy has persisting effects on asthma development in later life remains also uncertain. We acknowledge that residual confounding may remain after adjustment for offspring´s smoking status at age 31 years, since smoking status is likely to change over time. In this study the asthma diagnoses in early life were missing. Also the time of onset and duration of previous self-reported asthma or wheeze was not asked. It is only known that the onset and remission of these symptoms have occurred before the age of 3 years. We acknowledge that, despite the efforts in the definitions of a physician-diagnosed asthma, which is lung-function test -confirmed in our population, slight misclassification may exist. Our findings of interaction between RUNX1 haplotype and gestational smoke exposure in cumulative incidence of asthma between 31 years and 46 years of age, was based on small sample size and thus further studies on genome-environmental interaction and on gene expression level are mandatory.

Taken together, our study was able to demonstrate new evidence of an association between gestational smoke exposure and incidence of asthma and lung function in middle-aged offspring. Maternal smoking during pregnancy was associated with cumulative incidence of asthma in offspring between 31-46 years. The association was accentuated in offspring who reported at age 31 as having past respiratory problems and/or, who had haplotype rs11702779-AA. Also a reduction in FEV1/FVC ratio was observed at age 31 years in the

offspring with gestational smoke exposure. These results could reflect early vulnerability of offspring´s airways to ETS and its putative long-term effects. Our findings strengthen the previous hypothesis that maternal smoking has wide and long lasting consequences on airway health of offspring, and highlights the need to encourage women of childbearing age to permanently cease smoking.

Data sharing statement

No additional data available.

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Table 1. Association of maternal smoking during the last three months of pregnancy with cumulative incidence of physician-diagnosed asthma among offspring between the ages of 31 and 46 years. All subjects were free of physician-diagnosed asthma at age 31 years.

	Maternal smoking	n	% with asthma	OR_1 (95% CI)	p_1	OR_2 (95% CI)	p_2	p_i
Total	no	4727	6.8	1		1		
	yes	473	10.6	1.62 (1.18-2.21)	.003	1.54 (1.04-2.29)	.033	
Gender								
Men	no	2113	4.9	1		1		
	yes	201	7.0	1.45 (0.81-2.56)	.21	1.19 (0.57-2.47)	0.65	
Women	no	2614	8.3	1		1		
	yes	272	13.2	1.68 (1.15-2.45)	.007	1.71 (1.06-2.76)	.028	.45
Self-report of wheeze at baseline (age 31)								
Never wheeze	no	3386	5.1	1		1		
	yes	316	5.4	1.07 (0.64-1.78)	.80	1.04 (0.55-1.98)	.91	
$Current_3$ wheeze	no	483	14.5	1		1		
	yes	67	20.9	1.56 (0.82-2.96)	0.18	1.58 (0.71-3.54)	.26	.43
$Past_4$ wheeze	no	585	10.6	1		1		
	yes	58	27.6	3.21 (1.71-6.05)	<.001	2.62 (1.11-6.18)	.028	.075
Self-report of asthma at baseline (age 31)								
Never asthma	no	4548	6.3	1		1		
	yes	446	8.3	1.34 (0.94-1.91)	.11	1.26 (0.80-1.99)	.33	
Self-report of $current_3$ asthma	no	112	22.3	1		1		
	yes	16	37.5	2.09 (0.69-6.31)	.19	2.01 (0.45-9.05)	.36	.33

Self-report of past[4] asthma	no	52	15.4	1		1		
	yes	11	63.6	9.63 (2.28-40.67)	.002	37.86 (1.01-1417.65)	.049	.072

OR_1=crude odds ratio, OR_2= adjusted for selected covariates (mothers' education level, Apgar score at 1 minute, ponderal index at birth, parity, parental asthma, offspring's own education level at 31 years and offspring's smoking status at 31 years). p_i = adjusted p for interaction. [3]During the last 12 months. [4]Not during the last 12 months, but earlier. Adjusted p for interaction between Maternal smoking*Offspring´s smoking at age 31 was 0.32.

Table 2. Association of maternal smoking during the last three months of pregnancy with Spirometry values at the age of 31. All subjects were free of physician-diagnosed asthma at age 31 years.

	Maternal smoking	n	Median	IQR	$Model_1$ β	$Model_1$ 95% CI	$Model_1$ p	$Model_2$ β	$Model_2$ 95% CI	$Model_2$ p
Outcome: FEV1	no	3434	3.85	3.35-4.54	ref			ref		
	yes	341	3.73	3.26-4.47	-0.002	-0.06-0.05	.84	0.014	-0.02-0.09	.18
Outcome: FEV/FVC										
	no	3434	0.85	0.81-0.88	ref			ref		
	yes	341	0.83	0.79-0.87	-0.072	-0.02--0.01	<.001	-0.056	-0.02-0.01	.001

Spirometry data was available from 3775 offspring at 31 years of age. $Model_1$ = Adjusted by offspring's gender, height and weight at 31 years. $Model_2$ = Adjusted by offspring's gender, height and weight at 31 years, mothers' education level, Apgar score at 1 minute, ponderal index at birth, parity, parental asthma, offspring's own education level at 31 years and offspring's smoking status at 31 years. FEV1= Forced expiratory volume (Litres) during the first second, FEV1/FVC= the ratio between FEV1 and forced vital capacity (FVC). IQR=interquartile range (25^{th} percentile - 75^{th} percentile), β= standardized beta coefficient; P-values by linear regression model.

p for interaction:
Maternal smoking*FEV1 > Cumulative incidence of asthma between age 31 and 46; $p_i = 0.99$
Maternal smoking*FEV1/FVC ratio > Cumulative incidence of asthma between age 31 and 46; $p_i = 0.88$
p_i = adjusted by the variables of the $Model_2$

Table 3. Association of maternal smoking during the last three months of pregnancy with cumulative incidence of physician-diagnosed asthma among offspring between the ages of 31 and 46 years. All subjects free of physician-diagnosed asthma at age 31 years. The models are stratified by haplotype of the susceptibility genes and gender.

Gene, SNP	Maternal smoking	n	% with asthma	OR_1 (95% CI)	p	OR_2 (95% CI)	p	p_i
LSM14A, rs1759092[8]								
AA, men	no	327	4.9	1		1		
	yes	0	0.0	(0.00-∞)	1.00	- (0.00-∞)	1.00	1.00
AG, men	no	698	4.9	1		1		
	yes	65	10.8	2.36 (1.00-5.55)	.050	1.49 (0.54-4.09)	.44	1.00
GG, men	no	389	4.4	1		1		
	yes	29	6.9	1.62 (0.36-7.38)	.53	2.46 (0.40-15.05)	.33	1.00
AA, women	no	384	9.6	1		1		
	yes	50	12.0	1.30 (0.51-3.20)	.60	1.52 (0.58-3.98)	.40	.38
AG, women	no	850	6.5	1		1		
	yes	79	16.5	2.85 (1.48-5.48)	.002	3.04 (1.47-6.27)	**.003**	.33
GG, women	no	484	8.9	1		1		
	yes	53	13.2	1.56 (0.66-3.67)	.31	1.17 (0.41-3.38)	.77	.79
ADRB2, rs1042713								
AA, men	no	475	5.3	1		1		
	yes	38	13.2	2.73 (0.98-7.59)	.055	1.35 (0.37-4.96)	.65	.88
AG, men	no	685	4.7	1		1		
	yes	68	4.4	0.94 (0.28-3.16)	.92	0.81 (0.22-2.94)	.75	.62
GG, men	no	254	3.9	1		1		
	yes	20	5.0	1.28 (0.16-10.57)	.82	2.01 (0.15-26.75)	.60	.93
AA, women	no	520	6.3	1		1		
	yes	60	10.0	1.64 (0.66-4.09)	.29	1.76 (0.65-4.77)	.27	.68
AG, women	no	858	7.3	1		1		
	yes	91	16.5	2.49 (1.35-4.59)	.003	2.32 (1.17-4.59)	**.016**	.62

GG, women	no	340	11.5	1		1		
	yes	31	16.1	1.48 (0.54-4.09)	.45	1.27 (0.40-4.05)	.69	.72
IL3, rs40401								
TT, men	no	687	3.6	1		1		
	yes	59	10.2	3.00 (1.18-7.63)	.021	2.18 (0.75-6.30)	.15	.43
TC, men	no	589	5.4	1		1		
	yes	58	5.2	0.95 (0.28-3.20)	.93	0.68 (0.15-3.03)	.61	**.19**
CC, men	no	138	7.2	1		1		
	yes	9	0.0	0.00 (0.00-∞)	1.00	0.00 (0.00-∞)	1.00	1.00
AA, women	no	845	7.52	1		1		
	yes	83	15.7	2.31 (1.21-4.40)	.011	2.57 (1.29-5.11)	**.007**	.39
AG, women	no	724	7.7	1		1		
	yes	89	12.4	1.68 (0.85-3.35)	.14	1.12 (0.49-2.56)	.78	.21
GG, women	no	149	10.7	1		1		
	yes	10	20.0	2.08 (0.41-10.65)	.38	5.53 (0.73-41.96)	.098	.81
GSDMB, rs8069176$_4$								
AA, men	no	334	3.9	1		1		
	yes	32	3.1	0.80 (0.10-6.29)	.83	0.00 (0.00-∞)	1.00	.51
AG, men	no	691	4.8	1		1		
	yes	64	10.9	1.80 (0.83-3.90)	.14	2.25 (0.85-5.90)	.10	.30
GG, men	no	385	5.2	1		1		
	yes	30	3.3	0.63 (0.08-4.86)	.66	0.53 (0.07-4.31)	.55	.28
AA, women	no	412	8.0	1		1		
	yes	39	20.5	2.96 (1.26-6.97)	.013	2.20 (0.80-6.07)	.13	.49
AG, women	no	838	7.0	1		1		
	yes	97	13.4	1.69 (0.99-2.89)	.053	2.24 (1.14-4.41)	**.019**	.71
GG, women	no	464	9.3	1		1		
	yes	46	10.9	1.19 (0.45-3.18)	.72	0.91 (0.29-2.87)	.87	.50
GSDMB, rs4795400$_4$								
TT, men	no	329	4.0	1		1		
	yes	32	3.1	0.78 (0.10-6.20)	.82	0.00 (0.00-∞)	1.00	.52

TC, men	no	696	4.9	1		1		
	yes	63	11.1	2.43 (1.03-5.74)	.042	2.17 (0.82-5.67)	.12	1.00
CC, men	no	389	5.1	1		1		
	yes	31	3.2	0.62 (0.08-4.74)	.64	0.53 (0.07-4.34)	.56	1.00
AA, women	no	411	8.0	1		1		
	yes	38	18.4	2.59 (1.06-6.32)	.037	1.84 (0.63-5.39)	.27	.42
AG, women	no	838	7.0	1		1		
	yes	98	14.3	2.20 (1.18-4.11)	.013	2.42 (1.25-4.68)	**.009**	.89
GG, women	no	469	9.2	1		1		
	yes	46	10.9	1.21 (0.45-3.22)	.71	0.92 (0.29-2.90)	.89	.32
RUNX1, rs11702779								
AA, men	no	433	4.8	1		1		
	yes	45	2.2	0.45 (0.06-3.40)	.44	0.36 (0.04-3.05)	.35	.21
AG, men	no	667	4.8	1		1		
	yes	48	14.6	3.39 (1.41-8.14)	.006	2.28 (0.79-6.58)	.13	.11
GG, men	no	235	4.7	1		1		
	yes	28	3.6	0.75 (0.09-6.07)	.79	0.70 (0.07-6.94)	.76	.70
AA, women	no	572	6.3	1		1		
	yes	42	21.4	4.06 (1.81-9.13)	.001	5.53 (2.11-14.52)	**.001**	**.10**
AG, women	no	771	8.6	1		1		
	yes	95	13.7	1.69 (0.90-3.20)	.11	1.62 (0.79-3.29)	.19	.089
GG, women	no	280	10.0	1		1		
	yes	33	9.1	0.90 (0.26-3.14)	.87	0.98 (0.27-3.64)	.98	.054

OR_1=crude odds ratio, OR_2= adjusted for selected covariates (mothers' education level, Apgar score at 1 minute, ponderal index at birth, parity, parental asthma, offspring's own education level at 31 years and offspring's smoking status at 31 years). SNP= single nucleotide polymorphisms (SNP) of genes. p_i = adjusted p for interaction between gestational smoking exposure and gene haplotype in asthma risk. [4]SNPs that are in linkage disequilibrium. All the other SNPs were not in linkage disequilibrium.

Online data supplement

Maternal smoking during pregnancy affects adult onset of asthma in offspring: a follow up from birth to age 46 years

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Methods

The study population was based on the Northern Finland Birth Cohort in 1966 consisting of mothers and their children who were born in the northern Finland provinces of Oulu and Lapland in 1966 (1). The total number of births was 12058 in 1966, which covered 96.3% of all births in the area. In 1980, for the age 14 follow-up, 11764 alive subjects (97.6%) were traced and sent postal questionnaire, to which 11010 subjects responded. At the age of 31 in 1997, 10282 subjects were alive and traced. Among them, 8463 subjects who were still living in northern Finland or in the capital area were sent postal questionnaires and invited to clinical examinations to which 6025 subjects (71.2%) attended (2).

At the age of 46 in 2012-2013, 10300 subjects were alive and traced (85.4%). All subjects were given the opportunity to respond to two web based questionnaires (3,4) regarding background, lifestyle, health status, finances, work life and resources. If the participants did not have a computer, postal inquiries were sent to them. 7148 subjects completed data collection. The study was approved by the Ethics Committee of the Finnish Institute of Occupational Health and by the Ethics Committee of the Northern Ostrobothnia Hospital

District. At all stages of the study, the subjects gave written informed consent according to the Declaration of Helsinki.

Results

Exploring potential confounding factors and gender groups on offspring´s asthma onset between 31 and 46 years

When analysing the potential confounding factors, parental asthma affected the risk of offspring's asthma development between 31-46 years (Table 1, S1). Mothers' education, ponderal index (in tertile), Apgar score at the 1st minute and parity showed no significant effects on development of asthma between the ages of 31-46 years (Table 1, S1). Offspring´s education of 9 years or less or regular smoking at 31 years did not associate statistically significantly with asthma onset at 31-46 years (Table 1, S1). Mother´s age at the time of delivery did not associate with offspring's cumulative incidence of asthma between 31-46 years (OR=0.99 95% CI 0.97-1.00, p=0.076)

The effect of maternal smoking on the asthma onset of offspring with past respiratory problems

When analysing the associations of potential confounding factors with adult onset asthma in the offspring subgroups reporting asthma, parental asthma increased the risk of offspring's asthma development between 31-46 years, but only in the offspring subgroup reporting as never having had asthma, not diagnosed by a physician (Table 1, S2). Mothers' education, ponderal index (in tertile), Apgar score at the 1st minute showed no significant effects on development of asthma between the ages of 31-46 years in any subgroup (Table 1, S2). Parity associated with offspring´s onset of asthma between 31-46 years, in the subgroup who reported as having asthma, which is not doctor-diagnosed, at 31 years.

When analysing the potential confounding factors in the subgroups reporting wheeze, parental asthma affected the risk of offspring's asthma development between 31-46 years, but only in the offspring subgroup with beginning asthma at 31 years (Table 1, S3). Confounding factors showed no significant effects on development of asthma between the ages of 31-46 years in any subgroup (Table 1, S3). Parity associated with offspring´s onset of asthma between 31-46 years, in the subgroup with current wheeze at 31 years.

Discussion

Several SNPs have been identified as being susceptibility genes affecting asthma risk due to tobacco smoke exposure. These single nucleotide polymorphisms are found in loci of genes encoding glutathione S-transferase (GSTM1, GSTT1, GSTP1), Gasdermin (GSDMB, GSDML), ORMDL3, Sm protein family (LSM14A), Protein Tyrosine Phosphatase, Receptor (PTPRT), and Runt Related Transcription Factor 1 (RUNX1) (5-12). A study group found suggestive evidence for an interaction between rs8094633 on chromosome 18 near EPB41L3 and in utero tobacco smoke exposure and an interaction between rs1575472 on chromosome 6 in PACRG and childhood tobacco smoke exposure. The SNPs found have not been identified previously in general genome-wide association studies on childhood asthma. Interestingly, the SNPs interacting with in utero and childhood tobacco smoke exposure were different and were not involved in the same pathway (13). A study in a pediatric population showed that effects of in utero exposure to maternal smoking on childhood asthma and wheezing occurrence in offspring were largely restricted to children with the GSTM1-null genotype (8). GSTM1 functions in detoxification of electrophilic compounds, including carcinogens, therapeutic drugs, environmental toxins and products of oxidative stress, by conjugation with glutathione (8). In Finnish population the SNP of GSTM1 gene is rare and thus we were not able to study it. Yet the findings of our group and other groups could imply the impact of interaction between foetal genome and gestational environment on development of asthma in adulthood.

Regarding potential confounding factors, the observed effect could not be explained by covariates such as maternal education, ponderal index, Apgar score at the 1st minute, parity, parental asthma, offspring´s education and smoking at 31 years. However, the independent effect only remained significant among females. This could putatively reflect gender-based differences in utero and during postnatal life in programming airways towards asthma pathogenesis (14). Birth order associated with the cumulative incidence of asthma of adult offspring with current wheeze/beginning asthma so that if the birth order of the offspring was third, the

asthma risk was lower compared higher or lower birth order. This could be related to the offspring´s exposure of infections and airway development. Another study showed that among Israelii children who were the only child in the family, the prevalence of asthma was 7.3% (15). The prevalence increased to 8.95% among subjects from families with three siblings, and then progressively decreased as the number of siblings increased, and reached a trough of 0.58% in conscripts from families of 15 to 20 siblings. Asthma prevalence was similar for all birth orders (15).

A study in a Scottish population of pregnant women showed that the prevalence of self-reported current smoking (24 %) was significantly lower than a cotinine-validated estimate of current smokers (30 %) pointing out a need to offer smoking cessation programs to all pregnant women, in order to target pregnant women who do not disclose their smoking status (16). The proportion of pregnant smokers in our study was lower (9 %) than in previous studies in other populations probably in part due to differences in population and health care. As our interest was new diagnoses of adult asthma, we dropped off mothers whose offspring had had childhood asthma or suspicion of asthma before the age of 31 years. When we evaluated the whole cohort, the proportion of mothers who reported smoking during the last three months of pregnancy was increased. It must also be taken into account that in our study smoking status relied only on self-report to identify pregnant smokers, which underestimates the true number of pregnant smokers as previously reported (16). Furthermore the same study showed that the prevalence of smoking was higher (40 %) in mothers with the two highest categories indicating the biggest deprivation, according to the Scottish Index of Multiple Deprivation (16). We found that neither offspring´s education nor maternal education was associated with adult-onset asthma.

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Table S1. Cumulative incidence of physician-diagnosed asthma between the ages 31-46 years by maternal smoking during pregnancy, perinatal factors and selected factors at age 31 years - data from the 1966 Northern Finland Birth Cohort.

Factors	In total				Men				Women			
	All subjects n=5200	Subjects with new DD asthma n=372	% 9.1	P	All men n=2314	Subjects with new DD asthma n=118	% 5.1	P	All women n=2886	Subjects with new DD asthma n=254	% 8.8	P
Maternal smoking during the last three months of pregnancy												
No	4727	322	6.8	0.004	2113	104	4.9	0.237	2614	218	8.3	0.009
Yes	473	50	10.6		201	14	7.0		272	36	14.2	
Maternal smoking before the pregnancy												
No	4268	293	6.9	0.099	1893	90	4.8	0.167	2375	203	8.5	0.253
yes	885	75	8.5		402	26	6.5		483	49	10.1	
Number of cigarettes the mother smoked per day before pregnancy												
No	4727	322	6.8	0.011**	2113	104	4.9	0.133**	2614	218	8.3	0.012
1-5 cigarette	293	31	10.6		134	7	5.2		159	24	15.1	
>=6 cigarettes/pipes	180	19	10.6		67	7	10.4		113	12	10.6	
Change in smoking status during pregnancy												
No smoking	4265	292	6.8	0.113**	1892	90	4.8	0.279**	2373	202	8.5	0.035
Gave up	297	20	6.7		145	10	6.9		152	10	6.6	
Cut down	273	28	10.3		128	6	4.7		145	22	15.2	
No change or increased	306	27	8.8		123	10	8.1		183	17	9.3	

Ponderal index												
Lowest tertile	1736	136	7.8	0.274	851	49	5.8	0.566	885	87	9.8	0.319
Middle tertile	1711	110	6.4		741	34	4.6		970	76	7.8	
Highest tertile	1709	123	7.2		700	35	5.0		1009	88	8.7	
Apgar score at 1st minute												
<=6	149	13	8.7	0.520	83	7	8.4	0.203	66	6	9.1	0.829
7-10	4762	345	7.2		2098	108	5.1		2664	237	8.9	
Parity												
1	1641	121	7.4	0.582	736	33	4.5	0.505	905	88	9.7	0.454**
2	1274	101	7.9		586	35	6.0		688	66	9.6	
3	848	58	6.8		364	17	4.7		484	41	8.5	
4	469	33	7.0		220	15	6.8		249	18	7.2	
5 or more	961	59	6.1		404	18	4.5		557	41	7.4	
Maternal education level												
Matriculation examination	263	17	6.5	0.490	128	6	4.7	0.836	135	11	8.1	0.689
Secondary school or equivalent	1209	78	6.5		549	25	4.6		660	53	8.0	
Primary school or less	3682	274	7.4		1614	85	5.3		2068	189	9.1	
Parental asthma												
Neither	2885	187	6.5	0.01	1321	61	4.6	0.005	1564	126	8.1	0.059
Either or both	761	77	10.1		304	27	8.9		457	50	10.9	
Offspring´s education level at 31 years												
Matriculation examination	2322	158	6.8	0.447	764	34	4.5	0.365	1558	124	8.0	0.112
Less than matriculation examination	2859	211	7.4		1540	83	5.4		1319	128	9.7	

Offspring´s regular smoking at 31 years***												
No	4003	278	6.9	0.356	1671	78	4.7	0.194	2332	200	8.6	0.386
Yes	1111	86	7.7		608	37	6.1		503	49	9.7	

** trend test

*** smoking at least one cigarette per day for at least one year

Table S2. Cumulative incidence of physician-diagnosed asthma between the ages 31-46 years in three subgroups based on offspring´s self-reported never/beginning/past asthma without doctor-diagnosed asthma at 31 years. Analyzed by maternal smoking during pregnancy, perinatal factors and selected factors at age 31 years - data from the 1966 Northern Finland Birth Cohort.

Factors	Never asthma				Beginning asthma				Past asthma			
	n=4994	Subjects with new DD-asthma n=325	% 6.5	P	n=128	Subjects with new DD-asthma n=31	% 24.2	P	n=63	Subjects with new DD-asthma n=15	% 23.8	P
Maternal smoking during the last three months of pregnancy												
No	4548	288	6.3	0.108	112	25	22.3	0.215	52	8	15.4	0.002
Yes	446	37	8.3		16	6	37.5		11	7	63.6	
Maternal smoking before the pregnancy												
No	4112	261	6.3	0.146	101	24	23.8	1.00	42	7	16.7	0.120
yes	837	62	7.4		26	6	23.1		20	7	35.0	
Number of cigarettes the mother smoked per day before pregnancy												
No	4548	288	6.3	0.253**	112	25	22.3	0.290**	52	8	15.4	0.001**
1-5 cigarette	278	24	8.6		8	4	50.0		7	3	42.9	
>=6 cigarettes/pipes	168	13	7.7		8	2	25.0		4	4	100.0	
Change in smoking status during pregnancy												
No smoking	4110	261	6.4	0.474**	100	23	23.0	0.500**	42	7	16.7	0.007**
Gave up	279	18	6.5		11	2	18.2		6	0	0.0	
Cut down	260	23	8.8		6	3	50.0		6	2	33.3	
No change or increased	288	19	6.6		11	3	27.3		7	5	71.4	

Ponderal index												
Lowest tertile	1667	113	6.8	0.383	45	16	35.6	0.112	20	7	35.0	0.179
Middle tertile	1643	96	5.8		39	8	20.5		21	5	23.8	
Highest tertile	1644	114	6.9		42	7	16.7		20	2	10.0	
Apgar score at 1st minute												
<=6	140	11	7.9	0.493	6	2	33.3	0.631	3	0	0.0	0.561
7-10	4578	302	6.6		114	27	23.7		55	15	27.3	
Parity												
1	1575	99	6.3	0.614	37	15	40.5	0.031	25	7	28.0	0.166
2	1233	92	7.5		29	6	20.7		12	3	25.0	
3	811	51	6.3		26	2	7.7		8	4	50.0	
4	457	30	6.6		9	3	33.3		3	0	0.0	
5 or more	911	53	5.8		27	5	18.5		15	1	6.7	
Maternal education level												
Matriculation examination	252	17	6.7	0.694	5	0	0.0	0.390	5	0	0.0	0.621
Secondary school or equivalent	1171	70	6.0		24	5	20.8		13	3	23.1	
Primary school or less	3526	235	6.7		98	26	26.5		45	12	26.7	
Parental asthma												
Neither	2787	165	5.9	0.006	70	17	24.3	1.00	21	4	19.0	0.285
Either or both	709	63	8.9		32	7	21.9		18	7	38.9	
Offspring´s education level at 31 years												
Matriculation examination	2249	144	6.4	0.908	44	9	20.5	0.522	22	4	18.2	0.544
Less than matriculation examination	2726	178	6.5		84	22	26.2		41	11	26.8	

Offspring´s regular smoking at 31 years***												
No	3851	245	6.4	0.527	95	21	22.1	0.354	44	11	25.0	0.738
Yes	1060	73	6.9		33	10	30.3		17	3	17.6	

** trend test
*** smoking at least one cigarette per day for at least one year
DD= doctor-diagnosed

Table S3. Cumulative incidence of physician-diagnosed asthma between the ages 31-46 years in three subgroups based on offpring´s self-reported never/current/past wheeze at 31 years. Analyzed by maternal smoking during pregnancy, perinatal factors and selected factors at age 31 years - data from the 1966 Northern Finland Birth Cohort.

Factors	Never wheeze				Current wheeze				Past wheeze			
	n=3702	Subjects with new DD asthma n=188	% 5.1	P	n=550	Subjects with new DD asthma n=84	% 15.3	P	n=643	Subjects with new DD asthma n=78	% 12.1	P
Maternal smoking during the last three months of pregnancy												
No	3386	171	5.1	0.788	483	70	14.5	0.203	585	62	10.6	0.000
Yes	316	17	5.4		67	14	20.9		58	16	27.6	
Maternal smoking before the pregnancy												
No	3056	158	5.2	0.763	437	63	14.4	0.374	528	55	10.4	0.005
yes	613	29	4.7		111	20	18.0		107	22	20.6	
Number of cigarettes the mother smoked per day before pregnancy												
No	3386	171	5.1	0.978**	483	70	14.5	0.377**	585	62	10.6	0.002**
1-5 cigarette	197	11	5.6		42	9	21.4		36	10	27.8	
>=6 cigarettes/pipes	119	6	5.0		25	5	20.0		22	6	27.3	
Change in smoking status during pregnancy												
No smoking	3055	158	5.2	0.922**	437	63	14.4	0.528**	527	54	10.2	0.009**
Gave up	216	9	4.2		31	6	19.4		32	4	12.5	
Cut down	182	9	4.9		40	9	22.5		33	8	24.2	
No change or increased	210	10	4.8		39	6	15.4		41	10	24.4	

Ponderal index												
Lowest tertile	1220	66	5.4	0.823	194	33	17.0	0.587	223	28	12.6	0.706
Middle tertile	1226	60	4.9		168	22	13.1		206	22	10.78	
Highest tertile	1229	61	5.0		177	27	15.3		209	28	13.4	
Apgar score at 1st minute												
<=6	102	5	4.9	1.00	20	5	25.0	0.212	20	3	15.00	0.726
7-10	3392	1762	5.2		508	76	15.0		589	72	12.2	
Parity												
1	1173	57	4.9	0.455	175	35	20.0	0.027	209	22	10.5	0.795
2	903	56	6.2		123	16	13.0		161	23	14.3	
3	596	31	5.2		98	8	8.2		106	14	13.2	
4	337	14	4.2		44	11	23.0		62	8	12.9	
5 or more	6901	30	4.3		108	14	13.0		105	11	10.5	
Maternal education level												
Matriculation examination	184	11	6.0	0.646	27	2	7.4	0.391	39	4	10.3	0.596
Secondary school or equivalent	893	41	4.6		109	20	18.3		142	14	9.9	
Primary school or less	2593	134	5.2		410	62	15.3		457	60	13.1	
Parental asthma												
Neither	2119	105	5.0	0.309	292	36	12.3	0.003	353	39	11.0	0.307
Either or both	491	30	6.1		117	29	24.8		107	16	15.0	
Offspring´s education level at 31 years												
Matriculation examination	1725	91	5.3	0.908	199	31	15.6	0.902	282	29	10.3	0.271
Less than matriculation examination	1965	96	4.9		350	53	15.1		351	47	13.2	

Offspring´s regular smoking at 31 years***												
No	3006	151	5.0	0.619	317	56	17.7	0.070	496	57	11.5	1.00
Yes	632	35	5.5		227	27	11.9		137	16	11.7	

** trend test
*** smoking at least one cigarette per day for at least one year
DD= doctor-diagnosed

Table S4. List of the SNPs identified from a literature search associating with tobacco smoke exposure and asthma

SNP_id	PMID (REF)	chromosome: position	ext_gene_name	transcript_count	allele	MAF for ALL	MAF for AFR	MAF for AMR	MAF for EAS	MAF for FIN	MAF for NFE	MAF for OTH	MAF for SAS
rs1042713	18558635 (12)	5:148206156	ADRB2	1	G/A	2.968	3.372	3.066	2.940	3.988	3.022	2.617	2.797
rs11702779	21803869 (6)	21:36160098	RUNX1	19	G/A	2.601	2.467	3.706	2.851	3.154	2.788	2.503	2.684
rs1759092	28253294 (5)	19:34663409	LSM14A	8	G/A	4.094	4.692	3.481	3.794	1.101	3.788	4.184	4.053
rs2305480	22626592 (7)	17:38060848	GSDMB	15	G/A	2.440	0.985	2.243	2.920	1.810	3.562	3.047	3.160
rs366631	12186820 (8)	1:110252472	GSTM1	8	A/G	NA	NA	NA	NA	NA	NA	NA	NA
rs40401	24684517 (11)	5:131396222	IL3	1	C/T	2.413	3.643	1.765	2.024	3.678	2.213	1.677	2.015
rs4795400	22626592 (7)	17:38060848	GSDMB	15	C/T	2.477	1.106	2.235	2.920	1.803	3.562	3.056	3.182
rs7262414	28253294 (5)	20:40701392	PTPRT	8	C/A	1.082	0.715	0.638	0.966	0.0068	1.388	1.356	1.188
rs8069176	22626592 (7)	17:38060848	GSDMB	15	G/A	2.865	2.499	2.320	2.913	1.816	3.566	3.040	3.132
rs1695 (previous id rs947894)	20526719 (10)	11:67352689	GSTP1	1	A/G	0.354	0.450	0.375	0.172	0.270	0.335	0.317	NA
rs8076131	22626592 (7)	17:38060848	GSDMB	15	G/A	4.409	5.713	4.657	4.047	5.097	3.378	3.853	3.720

SNP=single nucleotide polymorphism, CHR: POS= chromosome position, ext=external, AFR=African, AMR=Admixed American, EAS=East Asian, FIN=Finnish, MAF = minor allele frequency, NFE=Non-Finnish European, OTH=Other, SAS=South Asian, NA=not applicable.