

Interaction between early maternal smoking and variants in *TNF* and *GSTP1* in childhood wheezing

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Summary

Background Children exposed to tobacco smoke early in life have a higher risk of wheeze. Individual susceptibility may depend on genetic factors.

Objective We studied whether variations in single nucleotide polymorphisms (SNPs) in the *TNF*, *glutathione S transferase P1 (GSTP1)* and *β2-adrenoreceptor (ADRB2)* genes modify the effect of early maternal smoking on the development of childhood asthma, wheeze and allergic sensitization.

Methods In the Swedish prospective birth cohort BAMSE (Children, Allergy, Milieu, Stockholm, Epidemiological Survey) ($n = 4089$), data collection included questionnaires to measure tobacco smoke exposure and clinical outcomes up to age 4 and medical examinations with blood sampling for specific IgE measurements and genotyping. We defined *early maternal smoking* as daily smoking by the mother during pregnancy and/or postnatally. We investigated five *TNF*, six *GSTP1* and three *ADRB2* SNPs in 982 selected wheezers and non-wheezers.

Results An interaction with early maternal smoking was found for three *TNF* SNPs (–857C/T, Intron 1, Intron 3) with respect to early wheeze (up to 2 years of age). For example, the odds ratio (OR) for developing early wheeze related to early maternal smoking was 2.4 [95% confidence interval (CI) 1.6–3.7] in children with a wild-type CC homozygote genotype of the *TNF* –857 SNP, while no tobacco-related risk was seen in children carrying the rare T allele. A clear dose response was observed in children with the CC genotype, with an OR of 1.3 (95% CI 1.1–1.5) per each additional pack per week smoked by the mother during pregnancy. A suggestive interaction with early maternal smoking was also seen for three *GSTP1* SNPs (Intron 5, Intron 6 and Ile105Val) with respect to transient wheeze, but not for *ADRB2* and wheeze phenotypes. No effect modifications were observed for allergic sensitization.

Conclusion Our results suggest that the risk of early childhood wheeze associated with early maternal smoking may be modified by *TNF* and *GSTP1* polymorphisms.

Keywords Asthma, children, environmental tobacco smoke, gene–environment interaction, *TNF*, wheeze

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Introduction

Exposure to tobacco smoke *in utero* and early in life leads to oxidative stress, as indicated in blood analysis in newborns and children at 3 months of age [1, 2]. Reactive oxygen species may induce inflammation. Tobacco-

exposed children have on average lower birth weight, impaired lung function and changed immune function parameters as well as a higher risk of developing wheeze and asthma [3–5]. The mechanisms leading to these effects are not fully clarified. Applying methods of genetic epidemiology may help in understanding the pathological

processes. Hypothetically, the response capacities of anti-oxidant and inflammatory systems in the developing child, defined by gene–gene and gene–environment interactions, determine the variability in individual phenotype.

Linkage studies indicate the presence of gene–environment interactions of smoking exposure with several chromosomal regions: 1p, 1q, 3p, 5q, 9q, 17p [6, 7]. A range of single nucleotide polymorphisms (SNPs) in xenobiotic-metabolizing enzyme genes (e.g. *CYP1A1*, *CYP1B1*, *GSTM1*) were found to interact significantly with smoking exposure in relation to asthma susceptibility [8, 9]. SNPs in other genes with yet unknown functions such as *GSDML/ORMDL3* in the 17q21 region have also been suggested to modify the effect of passive smoking [10]. Genes related to an antioxidant response [*glutathione S transferase P1* (*GSTP1*) located on chromosome 11q13], inflammation (TNF on 6p21) and bronchial hyperreactivity [β 2-adrenoreceptor (*ADRB2*) on 5q31–32] have been associated with asthma or atopy in previous studies and are also relevant candidate genes for environment interaction analyses [11, 12]. Few studies have examined the interactions of these genes with tobacco smoke exposure in children. The *GSTP1* coding SNP Ile105Val showed a significant interaction with *in utero* tobacco smoke exposure and exposure to air pollution in relation to wheeze and asthma [13, 14]. A number of *TNF* SNPs, e.g. *TNF* –308 and *TNF* –238, have been investigated in relation to modifying the effect of tobacco smoke exposure on asthma phenotypes in school-age children [15, 16]. The *ADRB2* Arg16Gly variation was reported to have a modifying effect of *in utero* and childhood tobacco smoke exposures on childhood wheezing [17]. Recently, results on gene–environment interactions with early exposure to air pollution have been reported, which support an effect modification by *GSTP1* Ile105Val and *TNF* –308 variants on the development of childhood allergy [18, 19]. Previous studies have thus pointed to the importance of the inflammation and antioxidative pathways and the involvement of candidate genes in the development of respiratory symptoms for modulating the response to environmental stimuli, including tobacco smoke, but the evidence is not conclusive.

In this study, we aimed to investigate whether variations in certain asthma candidate genes (*TNF*, *GSTP1* and *ADRB2*) modify the effect of early maternal smoking during pregnancy and/or postnatally on the development of asthma, wheeze and allergic sensitization in children up to 4 years.

Materials and methods

Study population and data collection procedures

The study was performed in the prospective birth cohort BAMSE (Children, Allergy, Milieu, Stockholm, Epidemiolo-

gical Survey) in Stockholm, Sweden, described elsewhere [20]. Briefly, 4089 newborn infants (75% of all eligible children in the selected Stockholm areas representing both urban and suburban districts) were included in BAMSE during the years 1994–1996.

Among the total cohort, 542 wheezing cases (see definitions below) and 542 random controls were selected for genetic analysis, based on availability of blood sample, questionnaire information and parental consent to genetic analyses [19]. We used the following case–cohort selection procedure: first, 709 children were randomly selected from all children with a blood sample ($n = 2298$), 542 of them being non-wheezers, which yielded a random sample of controls. The 167 wheezers from the random sample were complemented by all other identified wheezers ($n = 375$) in the cohort, forming a total group of 542 wheezing cases. After excluding subjects with failure in DNA extraction ($n = 29$) or <50% success rate for genotyping ($n = 73$), the final genetic analysis group included 982 children (497 wheezers and 485 controls). The study was performed in agreement with the Declaration of Helsinki and was approved by the ethical committee of Karolinska Institutet.

Data for the study were obtained by questionnaires and blood sample analyses. Parents completed questionnaires when their children were approximately 2 months, 1, 2 and 4 years of age. The questionnaires provided information on, e.g. wheeze episodes and asthma, maternal smoking during pregnancy and environmental tobacco smoke exposure, parental allergic disease and a range of other lifestyle factors relevant to the child's health.

Genotyping

DNA was extracted from peripheral blood leucocytes using a standard non-enzymatic method or the PURE-GENE KIT (Gentra Systems, Minneapolis, MN, USA) [19]. Analyses of SNPs in selected genes were performed with matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry (Sequenom, San Diego, CA, USA). The selected genetic variants for analysis included five *TNF* (*rs1799964*, *rs1799724*, *rs1800629*, *rs1800610*, *rs3093664*), six *GSTP1* (*rs762803*, *rs1695*, *rs749174*, *rs1138272*, *rs1871042*, *rs4891*) and three *ADRB2* (*rs1042714*, *rs1042717*, *rs1042718*) SNPs. More detailed information on the genotyping procedure has been published previously [19].

Exposure, outcome and confounder definitions

Early maternal smoking was considered if the mother smoked at least one cigarette per day at the time of the first questionnaire, when the child was approximately 2 months old (postnatal exposure), and/or smoked at least one cigarette per day at any time during pregnancy (*in utero*

exposure). We also defined three categories for the exposure to tobacco smoke during pregnancy (*in utero*) according to the highest reported number of cigarettes smoked by the mother per day during any of the pregnancy trimesters: 0 (reference group), 1–9 and >9 cigarettes per day.

Parental allergy was considered present if the mother and/or the father reported a doctor's diagnosis of asthma in combination with asthma medication and/or doctor-diagnosed hayfever in combination with allergy to furred pets and/or pollen allergy. *Socio-economic status* was defined as blue-collar worker, lower-level or medium-level white-collar worker, higher-level white-collar worker and other (including student, housewife/husband, disability pensioner and unemployed). *Exposure to environmental tobacco smoke* at ages 1, 2 and 4 years was considered present if any parent smoked at least one cigarette per day at the time of the respective questionnaire.

Wheeze phenotypes were determined in children followed up to 4 years based on questionnaire information. *Early-onset wheeze* was defined as wheeze up to 2 years of age, subdivided into *transient wheeze* (≥ 3 episodes of wheeze between 3 months and 2 years of age, but no episode in the last 12 months at 4 years) or *persistent wheeze* (≥ 1 episode of wheeze between 3 months and 2 years of age and ≥ 1 episode in the last 12 months at 4 years). *Late-onset wheeze* was defined as no episode of wheeze between 3 months and 2 years of age, but ≥ 1 episode in the last 12 months at 4 years [21]. *Current asthma* was defined as reported physician-diagnosed asthma up to 4 years of age and one or more episodes of wheezing in the last 12 months at 4 years. The reference group for analyses of asthma consisted of non-wheezing children without asthma diagnosis at 4 years.

Allergic sensitization was assessed with serum IgE tests for 2614 of the children at 4 years of age. It was analysed as specific IgE antibodies to inhalant and food allergens (Phadia AB, Uppsala, Sweden). The mix of inhalant allergens (Phadiatop[®]) included allergens of cat, dog, horse, birch, timothy, mugwort, *D. pteronyssinus* and *Cladosporium*, and the mix of food allergens (Fx5[®]) included allergens of milk, egg white, peanut, wheat, soy bean and fish. Sensitization to each category of allergens was defined as serum IgE antibody level ≥ 0.35 kilounits of antibody per litre (kU_A/L). In total, among the genotyped subjects, 266 children were included in the sensitized group, and we used 362 non-sensitized non-wheezers as a reference group. For sensitization, we also performed a sensitivity analysis focusing on the randomly selected BAMSE subcohort (167 wheezers and 542 non-wheezers), disregarding their wheezing status [19].

Statistical analysis

Logistic regression analysis was performed using STATA 9.0 (StataCorp, College Station, TX, USA) to estimate the

odds ratios (OR) with 95% confidence intervals (CI) for current asthma, wheeze and sensitization in relation to genetic factors and tobacco exposure. The main exposure variable used in the analysis was a dichotomous variable for early maternal smoking, supplemented by a dose-response variable with the three categories of *in utero* exposure defined above, as well as a continuous variable reflecting the average number of cigarette packs per week smoked by the mother during pregnancy. Likelihood ratio tests were used to assess the significance of gene–environment interaction parameters (0.05 as the significance level). We performed this analysis by comparing a model with maternal smoking and a genetic variant variable with a model also including an interaction term between those two. The interaction effects of gene polymorphisms and exposure were tested with both a dominant and a genotype-specific model [model-free/unconstrained genotype coding with indicator (dummy) variables for heterozygote and rare homozygote genotypes to investigate the pattern of effects across different genotypes]. For *TNF* interaction analyses, only dominant models were tested because of the few children with rare homozygote genotypes (due to a low rare allele frequency).

We present primarily the results of analyses performed in crude (unadjusted) models. We assessed the confounding effect of gender, mother's age, duration of exclusive breastfeeding, socio-economic status and environmental tobacco smoke exposure at ages 1, 2 and 4 years, and found that they did not affect the results appreciably. The results of adjusted models for early wheeze are presented in the supporting information. Because the study was designed to test specific hypotheses of interaction with known candidate genes, we present nominal test statistics. However, we also estimated the significance levels using Bonferroni's correction.

Results

The distribution of various exposure factors in relation to the phenotypes studied is shown in Table 1. The prevalence of early maternal smoking (during pregnancy and/or postnatally) was higher in wheezers than in non-wheezers, with the highest exposure prevalence among early-onset wheezers and children with diagnosed asthma.

In a model including only non-genetic risk factors, early maternal smoking in our study sample was significantly associated with an increased risk of early-onset wheeze (Table S1), with elevated point estimates also for current asthma and sensitization to airborne and food allergens. These results are in concordance with previous findings reported from the full BAMSE cohort [22].

Analysis of the main genetic effect showed significant associations for the *TNF* –308, *GSTP1* Intron 5 and *GSTP1* Ala114Val SNPs with current asthma, the *ADRB2*

Table 1. Descriptive characteristics of 982 genotyped children from the BAMSE cohort followed up to 4 years of age

	Asthma and wheeze-related phenotype				Sensitization-related phenotype	
	Current asthma <i>N</i> (%)	No wheeze <i>N</i> (%)	Early-onset wheeze <i>N</i> (%)	Late-onset wheeze <i>N</i> (%)	Non-sensitized non-wheezers* <i>N</i> (%)	Sensitization† <i>N</i> (%)
Total	136	485	366	131	362	266
Boys	86 (63.2)	227 (46.8)	217 (59.3)	78 (59.5)	161 (44.5)	159 (59.8)
Parental allergy	58 (43.3)	144 (30.0)	127 (35.5)	48 (36.9)	100 (27.9)	104 (39.7)
Exclusive breastfeeding ≥ 4 months	104 (76.5)	382 (78.8)	279 (76.4)	102 (78.5)	288 (79.6)	207 (77.8)
Mothers younger than 26 years	20 (14.7)	32 (6.6)	39 (10.7)	11 (8.4)	25 (6.9)	25 (9.4)
Socio-economic status						
Blue collar	33 (24.4)	86 (17.8)	58 (15.9)	18 (13.7)	61 (16.9)	44 (16.5)
Lower or medium white collar	73 (54.1)	205 (42.4)	177 (48.5)	65 (49.6)	155 (43.1)	132 (49.6)
Higher white collar	28 (20.7)	188 (38.9)	123 (33.7)	48 (36.6)	141 (39.2)	87 (32.7)
Other	1 (0.7)	4 (0.8)	7 (1.9)		3 (0.8)	3 (1.1)
Early maternal smoking (during pregnancy and/or at 2 months)	25 (18.4)	56 (11.6)	72 (19.7)	17 (13.0)	38 (10.5)	40 (15.0)
Maternal smoking only during pregnancy	10 (8.3)	22 (4.9)	34 (10.4)	6 (5.0)	15 (4.4)	15 (6.2)
Maternal smoking only at 2 months	1 (0.9)	2 (0.5)	4 (1.3)	1 (0.9)	1 (0.3)	2 (0.9)
ETS exposure at age 1 year	27 (19.9)	84 (17.4)	79 (21.6)	22 (16.9)	63 (17.4)	46 (17.4)
ETS exposure at age 2 years	33 (24.3)	77 (15.9)	86 (23.6)	21 (16.2)	58 (16.1)	46 (17.4)
ETS exposure at age 4 years	30 (22.4)	68 (14.1)	85 (23.4)	23 (17.7)	52 (14.5)	45 (17.0)

*Controls to the sensitized cases included non-sensitized non-wheezers.

†Sensitized cases (to inhalant or food allergens) were recruited from the whole sample of children.

Early maternal smoking was considered present if the mother smoked at least one cigarette per day at the time of enrolment (2 month's age of a child) and/or smoked at least one cigarette per day in any point of time during pregnancy.

Environmental tobacco smoke (ETS) exposure was considered present if any of the parents smoked at least one cigarette per day.

Current asthma was defined as reported physician-diagnosed asthma up to 4 years of age and one or more episodes of wheezing in the preceding 12 months at 4 years.

Early onset wheeze was defined as wheeze up to 2 years of age (having transient wheeze defined as ≥ 3 episodes of wheeze between 3 months and 2 years of age, but no episode in the last 12 months at 4 years; or persistent wheeze defined as ≥ 1 episode of wheeze between 3 months and 2 years of age and ≥ 1 episode in the last 12 months at 4 years).

Late-onset wheeze was defined as no episode of wheeze between 3 months and 2 years of age, but ≥ 1 episode in the last 12 months at 4 years.

Allergic sensitization was defined as serum IgE antibody level ≥ 0.35 kU_A/L.

Exon1 (rs1042717) SNP with early-onset wheeze and the *TNF* –308 SNP with allergic sensitization (Table S2).

Analysis of gene–environment interactions between the selected SNPs and early maternal smoking identified interactions for *TNF* SNPs in the promoter region (–857) and in Introns 1 and 3 for early-onset wheeze (Table 2). Adjustment for potential confounders only marginally altered the results (Table S3). No significant interactions were seen for late-onset wheeze, current asthma and sensitization to inhalant and food allergens (Table S4), or for individual allergens (data not shown). The sensitization results did not indicate any interaction of note, whether analysed with non-sensitized non-wheezers as controls or in the randomly selected subcohort of children.

The interaction models indicated a higher risk for developing early-onset wheeze related to early maternal smoking in wild-type CC homozygotes of the *TNF* SNPs –857 and Intron 1 (OR = 2.4, 95% CI 1.6–3.7 and OR = 2.5, 95% CI 1.6–3.8, respectively), while carriers of the rare T

allele of these SNPs did not experience an elevated risk (Table 2). For carriers of the rare G allele in the *TNF* Intron 3 SNP, the suggested increased risk was even greater (OR = 6.1, 95% CI 1.9–19.6), while common AA homozygotes had a less clear increase in risk. To gain further insight into the nature of association, we also performed a detailed analysis of *TNF* SNPs interactions in relation to transient and persistent wheeze phenotypes (Table S5), showing similar patterns of interaction for transient and persistent wheeze separately. *TNF* –857 and Intron 1 SNPs also showed a similar interaction pattern for current asthma, albeit not quite as strong and non-significant (e.g. $P = 0.10$ for the *TNF* –857 and current asthma; Table S4).

In the dose–response analysis based on the highest number of cigarettes smoked by the mother per day during any of the pregnancy trimesters [0 (reference), 1–9, and >9 cigarettes], carriers of the common *TNF* –857 and Intron 1 CC genotype showed a dose-dependent risk pattern ($P < 0.001$ for trend for both SNPs) and the

Table 2. Odds ratios (OR) for early-onset wheeze in children followed up to 4 years: effect modification of early maternal smoking exposure* by *TNF*, *GSTP1* and *ADRB2* gene variants

Phenotype	SNP	Gene and Locus	Minor allele frequency (%)	Genotype	Interaction analysis			
					Unexposed to smoking: Ca/Co [†]	Exposed to smoking: Ca/Co [†]	OR (95% CI) [‡] for maternal smoking exposure stratified by genotypes [§]	Interaction <i>P</i> -value
Early-onset wheeze <i>N</i> = 366	rs1799964	<i>TNF</i> -1031	21	TT	176/257	46/31	2.2 (1.3–3.6)	0.16
				TC/CC	98/142	19/23	1.2 (0.6–2.3)	
	rs1799724	<i>TNF</i> -857	9	CC	242/347	64/38	2.4 (1.6–3.7)	0.006
				CT/TT	46/73	6/17	0.6 (0.2–1.5)	
	rs1800629	<i>TNF</i> -308	16	GG	178/292	51/41	2.0 (1.3–3.2)	0.87
				GA/AA	90/115	19/11	2.2 (1.0–4.9)	
	rs1800610	<i>TNF</i>	9	CC	239/354	63/38	2.5 (1.6–3.8)	0.006
		Intron 1		CT/TT	48/70	7/17	0.6 (0.2–1.6)	
	rs3093664	<i>TNF</i>	8	AA	254/356	57/51	1.6 (1.0–2.4)	0.02
		Intron 3		AG/GG	39/63	15/4	6.1 (1.9–19.6)	
	rs762803	<i>GSTP1</i>	41	CC	102/157	17/19	1.4 (0.7–2.8)	0.61
		Intron 4		CA	130/196	37/29	1.9 (1.1–3.3)	
				AA	59/71	14/7	2.4 (0.9–6.4)	
	rs1695	<i>GSTP1</i>	33	AA (Ile)	131/190	24/24	1.5 (0.8–2.7)	0.17
		Exon 5, Ile105Val		AG (IleVal)	128/175	36/27	1.8 (1.1–3.2)	
				GG (Val)	30/51	10/3	5.7 (1.4–22.2)	
	rs749174	<i>GSTP1</i>	34	CC	124/193	23/26	1.4 (0.8–2.5)	0.09
		Intron 5		CT	137/174	33/25	1.7 (1.0–3.0)	
				TT	31/55	13/4	5.8 (1.7–19.2)	
	rs1138272	<i>GSTP1</i>	9	CC (Ala)	233/339	55/43	1.9 (1.2–2.9)	0.57
		Exon 6, Ala114Val		CT/TT (AlaVal)/(Val)	49/58	13/6	2.6 (0.9–7.3)	
	rs1871042	<i>GSTP1</i>	33	CC	130/198	25/26	1.5 (0.8–2.6)	0.07
		Intron 6		CT	129/170	32/25	1.7 (1.0–3.0)	
				TT	33/54	13/3	7.1 (1.9–26.8)	
	rs4891	<i>GSTP1</i>	30	TT	139/205	31/29	1.6 (0.9–2.7)	0.13
		Exon 7		TC	107/148	29/22	1.8 (1.0–3.3)	
				CC	31/49	12/3	6.3 (1.7–24.2)	
	rs1042714	<i>ADRB2</i>	41	CC (Gln)	97/150	23/17	2.1 (1.1–4.1)	0.72
		Exon 1, Gln27Glu		CG/GG (GlnGlu)/(Glu)	192/268	49/38	1.8 (1.1–2.9)	
	rs1042717	<i>ADRB2</i>	19	GG	196/254	51/33	2.0 (1.2–3.2)	0.67
		Exon 1		GA/AA	82/144	21/22	1.7 (0.9–3.2)	
	rs1042718	<i>ADRB2</i>	15	CC	213/295	54/34	2.2 (1.4–3.5)	0.28
		Exon 1		CA/AA	71/122	16/20	1.4 (0.7–2.8)	

*Early maternal smoking was considered present if the mother smoked at least one cigarette per day at the time of enrolment (2 month's age of a child) and/or smoked at least one cigarette per day in any point of time during pregnancy.

[†]Numbers of cases and controls.

[‡]Crude models.

[§]Unexposed to tobacco in each genotype category constitute the reference group for each OR.

Interaction effects with *TNF* and *ADRB2* SNPs were estimated in dominant genetic models. *GSTP1* SNPs were assessed with genotype-specific model, except for Ala114Val SNP, where dominant model was used because of few subjects with rare homozygote genotype.

Early-onset wheeze was defined as wheeze up to 2 years of age (having transient wheeze defined as ≥ 3 episodes of wheeze between 3 months and 2 years of age, but no episode in the last 12 months at 4 years; or persistent wheeze defined as ≥ 1 episode of wheeze between 3 months and 2 years of age and ≥ 1 episode in the last 12 months at 4 years).

highest risk of developing wheeze was detected in children exposed to >9 cigarettes per day (OR = 3.4, 95% CI 1.9–6.2; Fig. 1, Table S6, and OR = 3.4, 95% CI 1.9–6.2, respectively). A steeper but a less stable dose-response was seen for the Intron 3 SNP.

Such a dose response was not seen in the low-risk *TNF* genotype groups. In the continuous variable analysis, the OR for developing early-onset wheeze per each additional pack per week was 1.3 (95% CI 1.1–1.5) in children homozygous for the common –857 C allele, 1.3 (95% CI 1.1–1.5) in Intron 1 common homozygote CC subjects and 1.9 (95% CI 1.2–3.2) in Intron 3 variant allele G carriers, whereas no increase in risk was seen in the low-risk *TNF* genotype groups.

A statistically significant interaction with early maternal smoking was also noted for some *GSTP1* SNPs on transient wheeze (Table S5). Carriers of the rare T allele in the Introns 5 and 6 SNPs, or the Ile105Val G allele (coding for Val), who were exposed to early tobacco smoke, had an increased risk of developing transient wheeze, with the highest OR in children with a rare homozygote genotype (e.g. OR = 8.5, 95% CI 1.9–37.2 for *GSTP1* Ile105Val rare homozygote Val/Val carriers, Table S5).

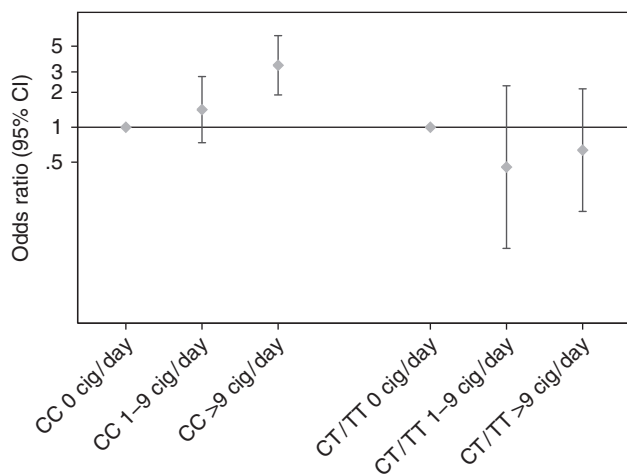


Fig. 1. Odds ratios for early-onset wheeze in children followed up to 4 years in relation to the extent of early maternal smoking during pregnancy (no, moderate: 1–9 cigarettes per day, and high: >9 cigarettes per day*), stratified by genetic variation in the *TNF* –857 SNP. *Corresponding to the highest number of cigarettes usually smoked per day by mother during any of the pregnancy trimesters. Early-onset wheeze was defined as wheeze up to 2 years of age (having transient wheeze defined as ≥ 3 episodes of wheeze between 3 months and 2 years of age, but no episode in the last 12 months at 4 years; or persistent wheeze defined as ≥ 1 episode of wheeze between 3 months and 2 years of age and ≥ 1 episode in the last 12 months at 4 years). There were 246 cases and 349 controls among unexposed CC carriers, 19 and 19 among CC carriers exposed to 1–9 cigarettes per day and 41 and 17 among CC carriers exposed to more than 9 cigarettes per day during pregnancy. The corresponding numbers for CT/TT carriers were 46 and 73 in unexposed, two and seven in exposed to 1–9 cigarettes and four cases and 10 controls in exposed to more than nine cigarettes per day (Table S6).

Discussion

Our analyses indicate the presence of gene–environment interactions between early maternal smoking and *TNF* and *GSTP1* polymorphisms with respect to the development of early-onset wheeze in children up to 4 years of age. The observed effect modification by the *TNF* promoter and intron SNPs has not been reported previously, which might indicate new areas of functional or regulatory importance in these genes. No clear interactions were found between the selected polymorphisms and early maternal smoking exposure with respect to current asthma (although the power was lower for this outcome), late-onset wheeze and sensitization.

TNF– α is a potent pro-inflammatory cytokine. Studies have shown that compounds in cigarette smoke are able to initiate gene transcription and the subsequent release of *TNF*– α from macrophages [23, 24]. Other studies report an inhibitory effect of cigarette smoke on *TNF*– α production and a functional unresponsiveness of T cells [25, 26], which may partly explain the anti-inflammatory properties of cigarette smoke. High neonatal cord-blood concentrations of *TNF*– α were associated with a lower risk of atopy, but not with wheezing or asthma risk in children at the age of 6 years [27]. In the same study, maternal smoking during pregnancy was associated with an increased risk of wheeze at 6 years, but not with cord-blood levels of *TNF*– α .

The *TNF* gene is located on chromosome 6p21 within the class III region of the human major histocompatibility complex. Certain *TNF* polymorphisms are shown to affect the transcriptional level of the gene; however, those findings are mostly not well confirmed [28]. Inconsistent results were reported for higher transcriptional activity of the rare –857T allele compared with the common –857C allele in different cell lines [29]. Despite the observed association of the ubiquitous transcription factor OCT-1 with the –857T allele in cell lines [30], suggesting activated transcription, a study in a Dutch population reported no effect of several *TNF* SNPs, including –857C/T, on *TNF* pre-mRNA production [28]. The discrepancy in the results may suggest the involvement of undetected regulatory factors both at transcriptional and post-transcriptional levels.

Few studies have addressed the question of an interaction between tobacco smoke exposure and *TNF* polymorphisms and consequent effects on respiratory symptoms in children, and those have focused mainly on the *TNF* –308 SNP. In a study of schoolchildren, the *TNF* –308G/A SNP modified the risk for respiratory illness-related school absences associated with second-hand smoke exposure, with –308A carriers exposed to tobacco smoke having a higher risk than unexposed homozygotes for the common –308G allele [15]. Another study reported effect modification by tobacco smoke exposure of

the association between *TNF* SNPs and childhood asthma. An increased risk of childhood asthma for the rare *TNF* –308A and –238A alleles was seen for children of non-smoking parents, while among children with smoking parents, none of the studied *TNF* polymorphisms were associated with asthma [16]. In the study of Li and colleagues, children with the common *TNF* –308 GG genotype had a reduced risk of asthma and wheezing. This effect appeared stronger in communities with low ozone exposure compared with high-ozone communities. The difference was more pronounced in *GSTM1* null and *GSTP1* Ile105Ile carriers, suggesting that the protective effect of the *TNF* –308 GG genotype depends on the level of antioxidative stress and the capacity of antioxidant defences [18]. In our study, we detected main genetic effects of the *TNF* –308G/A (rs1800629) polymorphism on current asthma and sensitization (Table S2), but the interaction with early maternal smoking was shown for other *TNF* variants, which, to our knowledge, were not investigated previously in terms of interaction with *in utero* tobacco smoke exposure. A clear dose-dependent effect was seen in children with the risk genotypes, which supports the biological relevance of our finding. The difference from the previous findings for the *TNF* –308 SNP could be explained by the following: the outcomes we used were related to wheeze, current asthma and allergic sensitization, the studied group included younger children and we considered *in utero* and postnatal tobacco smoke exposure. It is also possible that the variations in the *TNF* –857, Intron 1 and –308 SNPs may be linked to other true aetiologically important polymorphisms within the gene, with different linkage disequilibrium (LD) patterns in different populations. Our highly concordant results for the *TNF* –857 and Intron 1 SNPs are not surprising, because a high LD is observed between these SNPs ($r^2=0.93$) and they belong to the same haplotype block, while the Intron 3 SNP belongs to another block in the *TNF* gene (Fig. S1) [19]. The *TNF* –308 SNP belongs to the same LD block as the *TNF* –857 and Intron 1 SNPs, but is not so strongly associated with these SNPs (Fig. S1). The rare allele *TNF* –308A has been associated with an increased risk of childhood asthma and respiratory symptoms due to tobacco smoke exposure [15, 16], while in our study, the highest risk of early-onset wheeze is characteristic to common allele homozygotes of the *TNF* –857 and Intron 1 SNPs. Perhaps the mechanisms of tobacco smoke exposure effects *in utero* are more sensitive to *TNF* –857 and Intron 1 variation, while for exposed older children, *TNF* –308 variation plays a major role.

The *GSTP1* gene is located on chromosome 11q13 and is involved in antioxidant defence mechanisms. The difference in the host detoxification ability, including antioxidant defence, may determine the balance between resolution or progression of inflammation in atopic individuals after exposure to an environmental trigger, thus

leading to asthma eventually in only some atopic subjects [31]. Most studies have focused on the coding *GSTP1* Ile105Val SNP. Conflicting results were reported on the protective effect of the variant homozygote *GSTP1* Val105Val for allergy and asthma [19, 32, 33]. In the study of Li and colleagues, several *GSTP1* SNPs [rs6591255, rs4147581, rs1695 (Ile105Val) and rs749174] were investigated in terms of interaction with *in utero* tobacco smoke exposure and its effect on asthma and wheezing in children. Children with a Val105 allele and exposure to *in utero* tobacco smoke had about twice the risk of current wheezing and early-onset asthma as compared with Ile105Ile homozygote children without exposure [13]. The haplotype containing variant alleles for the *GSTP1* SNPs rs6591255, rs1695 (Ile105Val) and rs749174 had a protective effect for respiratory illness in tobacco smoke-unexposed schoolchildren. This protective effect was not observed in tobacco-exposed children [34]. A significant gene–environment interaction has been found for the Ile105Val polymorphism in Taiwanese children, where Ile105 homozygotes exposed to high levels of air pollution had a significantly higher risk of asthma compared with Val105 carriers exposed to low levels of air pollution [14]. A previous report from the BAMSE cohort found an increased risk of allergic sensitization in children with the *GSTP1* Val105 allele exposed to elevated levels of traffic-related air pollution compared with the risk in exposed Ile105 homozygotes [19]. In our study, we detected an indication for interactions between early maternal smoking and three of the *GSTP1* SNPs, including the Ile105Val SNP (Val carriers having the highest risk also in this study), on the development of wheeze, which supports a role of *GSTP1* in relation to environment exposures and asthma-related traits in children. Apart from the coding *GSTP1* Ile105Val SNP, we found some evidence for interaction in *GSTP1* SNPs in Introns 5 and 6. They are in the same LD block with the coding Ile105Val SNP (Fig. S1), and it is thus impossible to sort out any functional role of these SNPs based on these data.

Within the *ADRB2* gene, two functional SNPs (Arg16-Gly and Glu27Gln) have been investigated for interaction with *in utero* and childhood second-hand tobacco smoke exposure and its effect on asthma and wheezing in children. Children homozygous for the Arg16 allele and exposed to maternal smoking *in utero* had a higher risk of lifetime wheeze compared with unexposed Gly16 carriers [17]. In ever-smoking adults, the presence of the Arg 16Arg genotype was associated with an increased risk of asthma compared with never-smoking Gly16Gly carriers. [35] Our study did not find any associations for the *ADRB2* SNPs in Exon 1, including Glu27Gln SNP.

In several cases, significant interaction *P*-values were seen in our study despite a non-significant main genetic effect (for interaction analysis, see Table 2 and Table S4; for main genetic effects, see Table S2). On the other hand,

despite the presence of a significant main genetic effect, the *TNF* –308 SNP did not show a statistically significant interaction for current asthma or sensitization.

The marginal effects of genetic variants are often overpowered by the influence of an environmental factor, leading to more pronounced gene–environment interaction effects [14, 17, 36]. One may assume that the increasing trends of allergy and asthma are most likely due to changes in the environment, which uncover pre-existing genetic susceptibilities [12]. Such asthma-associated environmental factors may include exposure to environmental tobacco smoke, air pollution, a low-antioxidant diet or respiratory virus infections [12]. It is usually suggested that such exposures (e.g. tobacco smoke exposure) should be taken into account when evaluating the role of genetic variants and disease risk [17, 36].

For a more thorough investigation of the pathological mechanisms of *in utero* tobacco smoke exposure, performing measurements of the intermediate phenotype (e.g. *TNF*- α levels at birth) and of biomarkers of exposure (e.g. cotinine levels) would be useful. Such analyses were not available in our study. Despite the relatively large numbers of wheezing children and controls, we still faced power limitations due to the relatively few rare homozygotes in *TNF* SNPs. The gene–environment interactions observed are not significant globally after multiple test correction (Bonferroni's test). Although the Bonferroni's test is very conservative, this may reflect inadequate power, the fact that the interaction effects are rather small or false-positive findings. We believe, however, that global multiple-testing procedures are inappropriate in our study setting, as this study does not engage in hypothesis-free testing across the genetic or the environmental variable space. This longitudinal study investigates specific hypotheses. Previous studies support *TNF* as an effect modifier of passive smoking effects and our dose-dependent results indicate that false-positive results are unlikely. The most reliable confirmation of a true effect would be replications of the detected associations in unrelated study groups, as well as the experimental confirmation of functional changes due to discovered SNPs or functional SNPs linked to them. Among other limitations of the study is the lack of information on mothers' passive smoking during pregnancy. Regarding postnatal exposures, a more detailed research on the effect of viral infections on the development of wheezing would also be of interest in order to distinguish the two major mechanisms of asthma pathogenesis in this age group: inflammation triggered by viral infections or atopic mechanisms. Based on the selection of children for genotyping (wheezers and non-wheezing controls), we might face a potential selection bias in the analysis of other outcomes (e.g. sensitization), as our sample is over-representative for wheezing children. However, the alternative sensitivity analysis in the group of randomly selected children

addresses this and in fact did not materially change the results for the sensitization outcome.

The strengths of the study, apart from being performed in a large birth cohort representative of the Stockholm population, include the information on tobacco smoke exposure collected repeatedly before any adverse health symptoms (wheeze and sensitization) occurred. The data collected on a wide range of potential confounders facilitate a study of the factors involved in pathogenesis and interaction with the main exposure. The proportion of children exposed to early maternal smoking among those genotyped in this study was similar to that reported previously in the overall BAMSE cohort [37], which makes our group representative in terms of exposure assessment. The analysis of the main effects of early tobacco smoke exposure performed in our sample also agrees with the overall BAMSE findings for wheeze [38].

Although the majority of children with early wheeze will outgrow their symptoms [39], these children have been shown to have lower levels of lung function during childhood and adolescence [40]. Thus, early determinants for both wheeze susceptibility and lung function capacity, such as *TNF* variants and tobacco smoke exposure described in this study, seem to be of importance for respiratory status later in life. The fact that early maternal smoking was found to increase the risk of early wheeze (and possibly current asthma) in carriers of common alleles in the *TNF* SNPs (~83% of children, i.e. a large majority) supports the general recommendations for smoking cessation and avoiding tobacco smoke exposure for pregnant women and children. The observed interactions also provide additional evidence for the involvement of *TNF*- α and *GSTP1* in pathological changes occurring due to early tobacco smoke exposure. Further research could extend these findings by addressing issues of the functional activity of *TNF* and *GSTP1* SNPs, as well as the type and critical time intervals of environmental exposures that may be of particular importance for certain asthma-related phenotypes.

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Conflict of interest: Fredrik Nyberg is employed by AstraZeneca and AstraZeneca also supports his academic part-time adjunct position as Lecturer in Molecular Epidemiology at Karolinska Institutet.

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Supporting Information

Additional supporting information may be found in the online version of this article:

Table S1. Odds ratios for current asthma, wheeze and allergic sensitization in children up to 4 years in relation to early maternal smoking*.

Table S2. Odds Ratios for current asthma, early-onset wheeze, late onset wheeze and allergic sensitization* in children followed up to 4 years: main genetic effects of *TNF*, *GSTP1* and *ADRB2* SNPs.

Table S3. Adjusted Odds Ratios for early-onset wheeze in children followed up to 4 years: effect modification of early maternal smoking exposure* by *TNF*, *GSTP1* and *ADRB2* gene variants.

Table S4. Odds Ratios for current asthma, late-onset wheeze and sensitization in children followed up to 4 years: effect modification of early maternal smoking exposure* by *TNF*, *GSTP1* and *ADRB2* gene variants.

Table S5. Odds Ratios for transient and persistent wheeze in children followed up to 4 years: effect modification of early maternal smoking exposure* by *TNF* and *GSTP1* gene variants.

Table S6. Odds ratios for early-onset wheeze in children followed up to 4 years in relation to extent of maternal smoking during pregnancy (non smoking, moderate: 1–9 cigarettes per day, and high: >9 cigarettes per day*), stratified by genetic variation in the *TNF* -857 (rs1799724) SNP.

Figure S1. Block structure and correlation of the SNPs in the *TNF* gene (A), *GSTP1* gene (B), and *ADRB2* gene (C). The numbers in each box correspond to the pairwise correlation coefficient r^2 between respective SNPs. Defining the block structure was based on the linkage disequilibrium coefficient D' using Haploview (Solid spine of LD).

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SUPPLEMENTARY MATERIAL

Table S1. Odds ratios for current asthma, wheeze and allergic sensitisation in children up to 4 years in relation to early maternal smoking*.

Phenotype	Crude models			Adjusted models†		
	Unexposed to smoking Ca / Co ‡	Exposed to smoking Ca / Co‡	Main effect of maternal smoking OR (95% CI)	Unexposed to smoking Ca / Co‡	Exposed to smoking Ca / Co‡	Main effect of maternal smoking OR (95% CI)
Current asthma	111/429	25/56	1.7 (1.0-2.9)	108/423	25/55	1.5 (0.7-2.9)
Early onset wheeze	294/429	72/56	1.9 (1.3-2.7)	287/423	72/55	1.7 (1.0-2.8)
Late onset wheeze	114/429	17/56	1.1 (0.6-2.0)	110/423	17/55	1.3 (0.6-2.9)
Sensitisation	226/324	40/38	1.5 (0.9-2.4)	222/320	40/37	1.8 (1.0-3.4)

* Early maternal smoking was considered present if the mother smoked at least one cigarette per day at the time of enrolment (2 month's age of a child) and/or smoked at least one cigarette per day in any point of time during pregnancy.

Current asthma was defined as reported physician-diagnosed asthma up to 4 years of age and one or more episodes of wheezing in the preceding 12 months at 4 years.

Early onset wheeze was defined as wheeze up to 2 years of age (having transient wheeze defined as ≥ 3 episodes of wheeze between 3 months and 2 years of age, but no episode in the last 12 months at 4 years; or persistent wheeze defined as ≥ 1 episode of wheeze between 3 months and 2 years of age and ≥ 1 episode in the last 12 months at 4 years).

Late-onset wheeze was defined as no episode of wheeze between 3 months and 2 years of age, but ≥ 1 episode in the last 12 months at 4 years.

Allergic sensitisation was defined as serum IgE antibody level ≥ 0.35 kU_A/l.

† Adjusted for sex, mother's age, breast feeding, socioeconomic status, environmental tobacco smoke exposure at 1, 2 and 4 years.

‡ Numbers of cases and controls.

Table S2. Odds Ratios for current asthma, early onset wheeze, late onset wheeze and allergic sensitisation* in children followed up to 4 years: main genetic effects of *TNF*, *GSTP1* and *ADRB2* SNPs.

Pheno- type	SNP	Gene and Locus	Minor allele frequency, %	Genotype	Main genetic effect analysis	
					Ca / Co†	OR (95% CI)‡ for genotype
Current asthma N=136	rs1799964	<i>TNF</i> -1031	21	TT	82/288	1.0 (ref.)
				TC/CC	44/165	0.9 (0.6-1.4)
	rs1799724	<i>TNF</i> -857	9	CC	111/385	1.0 (ref.)
				CT/TT	22/90	0.8 (0.5-1.4)
	rs1800629	<i>TNF</i> -308	16	GG	80/333	1.0 (ref.)
				GA/AA	49/126	1.6 (1.1-2.4)
	rs1800610	<i>TNF</i> Intron 1	9	CC	111/392	1.0 (ref.)
				CT/TT	23/87	0.9 (0.6-1.5)
	rs3093664	<i>TNF</i> Intron 3	8	AA	118/407	1.0 (ref.)
				AG/GG	17/67	0.9 (0.5-1.5)
	rs762803	<i>GSTP1</i> Intron 4	41	CC	43/176	1.0 (ref.)
				CA	66/225	1.2 (0.8-1.8)
				AA	26/78	1.4 (0.8-2.4)
	rs1695	<i>GSTP1</i> Exon 5, Ile105Val	33	AA (Ile)	55/214	1.0 (ref.)
				AG (Ile/Val)	68/202	1.3 (0.9-2.0)
				GG (Val)	11/54	0.8 (0.4-1.6)
	rs749174	<i>GSTP1</i> Intron 5	34	CC	48/219	1.0 (ref.)
				CT	75/199	1.7 (1.1-2.6)
				TT	12/59	0.9 (0.5-1.9)
	rs1138272	<i>GSTP1</i> Exon 6, Ala114Val	9	CC (Ala)	98/382	1.0 (ref.)
				CT/TT (AlaVal)/(Val)	34/64	2.1 (1.3-3.3)
	rs1871042	<i>GSTP1</i> Intron 6	33	CC	54/224	1.0 (ref.)
				CT	67/195	1.4 (0.9-2.1)
				TT	14/57	1.0 (0.5-2.0)
	rs4891	<i>GSTP1</i> Exon 7	30	TT	61/234	1.0 (ref.)
				TC	55/170	1.2 (0.8-1.9)
				CC	12/52	0.9 (0.4-1.8)
	rs1042714	<i>ADRB2</i> Exon 1, Gln27Glu	41	CC (Gln)	49/167	1.0 (ref.)
				CG/GG (GlnGlu)/(Glu)	86/306	1.0 (0.6-1.4)
	rs1042717	<i>ADRB2</i> Exon 1	19	GG	90/287	1.0 (ref.)
				GA/AA	41/166	0.8 (0.5-1.2)
	rs1042718	<i>ADRB2</i> Exon 1	15	CC	98/329	1.0 (ref.)
				CA/AA	35/142	0.8 (0.5-1.3)
Early onset wheeze N=366	rs1799964	<i>TNF</i> -1031	21	TT	222/288	1.0 (ref.)
				TC/CC	117/165	0.9 (0.7-1.2)
	rs1799724	<i>TNF</i> -857	9	CC	306/385	1.0 (ref.)
				CT/TT	52/90	0.7 (0.5-1.1)
	rs1800629	<i>TNF</i> -308	16	GG	229/333	1.0 (ref.)
				GA/AA	109/126	1.3 (0.9-1.7)
	rs1800610	<i>TNF</i> Intron 1	9	CC	302/392	1.0 (ref.)
				CT/TT	55/87	0.8 (0.6-1.2)
	rs3093664	<i>TNF</i> Intron 3	8	AA	311/407	1.0 (ref.)
				AG/GG	54/67	1.1 (0.7-1.6)
	rs762803	<i>GSTP1</i> Intron 4	41	CC	119/176	1.0 (ref.)
				CA	167/225	1.1 (0.8-1.5)
				AA	73/78	1.4 (0.9-2.1)

Pheno- type	SNP	Gene and Locus	Minor allele frequency,	Genotype	Main genetic effect analysis	
					Ca / Co†	OR (95% CI)‡ for genotype
Early onset wheeze N=366	rs1695	<i>GSTP1</i> Exon 5, Ile105Val	33	AA (Ile)	155/214	1.0 (ref.)
				AG (Ile/Val)	164/202	1.1 (0.8-1.5)
				GG (Val)	40/54	1.0 (0.6-1.6)
	rs749174	<i>GSTP1</i> Intron 5	34	CC	147/219	1.0 (ref.)
				CT	170/199	1.3 (0.9-1.7)
				TT	44/59	1.1 (0.7-1.7)
				CT/TT	288/382	1.0 (ref.)
	rs1138272	<i>GSTP1</i> Exon 6, Ala114Val	9	CC (Ala)	62/64	1.3 (0.9-1.9)
				(AlaVal)/(Val)		
	rs1871042	<i>GSTP1</i> Intron 6	33	CC	155/224	1.0 (ref.)
				CT	161/195	1.2 (0.9-1.6)
				TT	46/57	1.2 (0.8-1.8)
	rs4891	<i>GSTP1</i> Exon 7	30	TT	170/234	1.0 (ref.)
				TC	136/170	1.1 (0.8-1.5)
				CC	43/52	1.1 (0.7-1.8)
	rs1042714	<i>ADRB2</i> Exon 1, Gln27Glu	41	CC (Gln)	120/167	1.0 (ref.)
				CG/GG	241/306	1.1 (0.8-1.5)
	rs1042717	<i>ADRB2</i> Exon 1	19	GG	247/287	1.0 (ref.)
				GA/AA	103/166	0.7 (0.5-1.0)
	rs1042718	<i>ADRB2</i> Exon 1	15	CC	267/329	1.0 (ref.)
				CA/AA	87/142	0.8 (0.6-1.0)
Late onset wheeze N=131	rs1799964	<i>TNF</i> -1031	21	TT	72/288	1.0 (ref.)
	rs1799724	<i>TNF</i> -857	9	TC/CC	51/165	1.2 (0.8-1.9)
				CC	109/385	1.0 (ref.)
				CT/TT	19/90	0.7 (0.4-1.3)
	rs1800629	<i>TNF</i> -308	16	GG	87/333	1.0 (ref.)
				GA/AA	30/126	0.9 (0.6-1.4)
	rs1800610	<i>TNF</i> Intron 1	9	CC	109/392	1.0 (ref.)
				CT/TT	18/87	0.7 (0.4-1.3)
	rs3093664	<i>TNF</i> Intron 3	8	AA	107/407	1.0 (ref.)
				AG/GG	19/67	1.1 (0.6-1.9)
	rs762803	<i>GSTP1</i> Intron 4	41	CC	55/176	1.0 (ref.)
				CA/AA	73/303	0.8 (0.5-1.1)
	rs1695	<i>GSTP1</i> Exon 5, Ile105Val	33	AA (Ile)	65/214	1.0 (ref.)
				AG/GG	62/256	0.8 (0.5-1.2)
				(Ile/Val)/(Val)		
	rs749174	<i>GSTP1</i> Intron 5	34	CC	56/219	1.0 (ref.)
				CT/TT	70/258	1.1 (0.7-1.6)
	rs1138272	<i>GSTP1</i> Exon 6, Ala114Val	9	CC (Ala)	104/382	1.0 (ref.)
				CT/TT	23/64	1.3 (0.8-2.2)
	rs1871042	<i>GSTP1</i> Intron 6	33	CC	66/224	1.0 (ref.)
				CT/TT	63/252	0.8 (0.6-1.3)
	rs4891	<i>GSTP1</i> Exon 7	30	TT	68/234	1.0 (ref.)
				TC/CC	59/222	0.9 (0.6-1.4)
	rs1042714	<i>ADRB2</i> Exon 1, Gln27Glu	41	CC (Gln)	50/167	1.0 (ref.)
				CG/GG	78/306	0.9 (0.6-1.3)
	rs1042717	<i>ADRB2</i> Exon 1	19	(GlnGlu)/(Glu)		
				GG	73/287	1.0 (ref.)
	rs1042718	<i>ADRB2</i> Exon 1	15	GA/AA	50/166	1.2 (0.8-1.8)
				CC	86/329	1.0 (ref.)
				CA/AA	43/142	1.2 (0.8-1.8)

Pheno- type	SNP	Gene and Locus	Minor allele frequency,	Genotype	Main genetic effect analysis	
					Ca / Co†	OR (95% CI)‡ for genotype
Sensiti- sation N=266	rs1799964	<i>TNF</i> -1031	21	TT	151/218	1.0 (ref.)
	rs1799724	<i>TNF</i> -857	9	TC/CC	97/122	1.1 (0.8-1.6)
				CC	216/292	1.0 (ref.)
				CT/TT	46/63	1.0 (0.6-1.5)
	rs1800629	<i>TNF</i> -308	16	GG	163/263	1.0 (ref.)
				GA/AA	85/80	1.7 (1.2-2.5)
	rs1800610	<i>TNF</i>	9	CC	215/296	1.0 (ref.)
	rs3093664	Intron 1	8	CT/TT	46/62	1.0 (0.7-1.6)
		<i>TNF</i>		AA	225/301	1.0 (ref.)
	rs762803	Intron 3	41	AG/GG	36/53	0.9 (0.6-1.4)
		<i>GSTP1</i>		CC	91/132	1.0 (ref.)
		Intron 4		CA	122/167	1.1 (0.7-1.5)
	rs1695	<i>GSTP1</i>	33	AA	50/58	1.3 (0.8-2.0)
				AA (Ile)	112/161	1.0 (ref.)
				AG (Ile/Val)	122/148	1.2 (0.8-1.7)
	rs749174	<i>GSTP1</i>	34	GG (Val)	26/43	0.9 (0.5-1.5)
				CC	113/158	1.0 (ref.)
				CT	120/150	1.1 (0.8-1.6)
	rs1138272	<i>GSTP1</i>	9	TT	29/48	0.8 (0.5-1.4)
				CC (Ala)	207/285	1.0 (ref.)
				CT/TT	41/47	1.2 (0.8-1.9)
	rs1871042	<i>GSTP1</i>	33	(AlaVal)/(Val)		
				CC	116/163	1.0 (ref.)
				CT	119/146	1.1 (0.8-1.6)
	rs4891	<i>GSTP1</i>	30	TT	28/47	0.8 (0.5-1.4)
				TT	121/173	1.0 (ref.)
				TC	103/128	1.2 (0.8-1.6)
	rs1042714	<i>ADRB2</i>	41	CC	26/42	0.9 (0.5-1.5)
				CC (Gln)	105/116	1.0 (ref.)
				CG/GG	156/237	0.7 (0.5-1.0)
	rs1042717	<i>ADRB2</i>	19	(GlnGlu)/(Glu)		
				GG	156/220	1.0 (ref.)
	rs1042718	<i>ADRB2</i>	15	GA/AA	92/120	1.1 (0.8-1.5)
				CC	179/249	1.0 (ref.)
		Exon 1		CA/AA	79/104	1.1 (0.7-1.5)

* Current asthma was defined as reported physician-diagnosed asthma up to 4 years of age and one or more episodes of wheezing in the preceding 12 months at 4 years.

Early onset wheeze was defined as wheeze up to 2 years of age (having transient wheeze defined as ≥ 3 episodes of wheeze between 3 months and 2 years of age, but no episode in the last 12 months at 4 years; or persistent wheeze defined as ≥ 1 episode of wheeze between 3 months and 2 years of age and ≥ 1 episode in the last 12 months at 4 years).

Late-onset wheeze was defined as no episode of wheeze between 3 months and 2 years of age, but ≥ 1 episode in the last 12 months at 4 years.

Allergic sensitisation was defined as serum IgE antibody level ≥ 0.35 kU_A/l.

† Numbers of cases and controls.

‡ Crude models.

Genetic effects of *TNF* and *ADRB2* SNPs were estimated in dominant genetic models. *GSTP1* SNPs were assessed with genotype-specific model, except for Ala114Val SNP and late-onset wheeze estimates, where dominant model was used because of few subjects with rare homozygote genotype.

Table S3. Adjusted Odds Ratios for early onset wheeze in children followed up to 4 years: effect modification of early maternal smoking exposure* by *TNF*, *GSTP1*, and *ADRB2* gene variants.

Pheno- type	SNP	Gene and Locus	Minor allele frequent cy, %	Genotype	Interaction analysis			
					Unexposed to smoking: Ca / Co†	Exposed to smoking: Ca / Co†	OR (95% CI)‡ for maternal smoking exposure stratified by genotypes§	Intera ction p- value
Early onset wheeze N=366	rs1799964	<i>TNF</i> -1031	21	TT	172/251	46/30	2.1 (1.1-3.8)	0.10
				TC/CC	97/142	19/23	1.0 (0.5-2.1)	
	rs1799724	<i>TNF</i> -857	9	CC	236/344	64/37	2.2 (1.2-3.7)	0.01
				CT/TT	45/70	6/17	0.5 (0.2-1.5)	
	rs1800629	<i>TNF</i> -308	16	GG	175/288	51/41	1.9 (1.1-3.3)	0.85
				GA/AA	87/113	19/10	2.1 (0.8-5.1)	
	rs1800610	<i>TNF</i> Intron 1	9	CC	233/351	63/37	2.2 (1.3-3.8)	0.008
				CT/TT	47/67	7/17	0.5 (0.2-1.5)	
	rs3093664	<i>TNF</i> Intron 3	8	AA	247/350	57/50	1.4 (0.9-2.5)	0.03
				AG/GG	39/63	15/4	5.5 (1.6-18.9)	
	rs762803	<i>GSTP1</i> Intron 4	41	CC	101/152	17/19	1.3 (0.6-2.8)	0.60
				CA	126/196	37/29	1.8 (0.9-3.3)	
				AA	57/70	14/6	2.4 (0.8-7.3)	
	rs1695	<i>GSTP1</i> Exon 5,	33	AA (Ile)	129/185	24/24	1.2 (0.6-2.5)	0.17
				AG (Ile/Val)	124/175	36/26	1.8 (0.9-3.5)	
	rs749174	<i>GSTP1</i> Intron 5	34	GG (Val)	29/50	10/3	5.0 (1.2-21.0)	0.07
				CC	123/188	23/26	1.2 (0.6-2.3)	
				CT	132/174	33/24	1.7 (0.8-3.3)	
	rs1138272	<i>GSTP1</i> Exon 6, Ala114Val	9	TT	30/54	13/4	5.4 (1.5-19.6)	0.74
				CC (Ala)	228/334	55/42	1.7 (1.0-2.9)	
				CT/TT (AlaVal)/ (Val)	47/57	13/6	2.0 (0.7-6.2)	
	rs1871042	<i>GSTP1</i> Intron 6	33	CC	127/193	25/26	1.3 (0.7-2.5)	0.06
				CT	126/170	32/24	1.6 (0.8-3.2)	
				TT	32/53	13/3	6.9 (1.7-27.9)	
	rs4891	<i>GSTP1</i> Exon 7	30	TT	137/200	31/29	1.3 (0.7-2.5)	0.14
				TC	104/148	29/21	1.8 (0.9-3.6)	
				CC	30/48	12/3	5.6 (1.4-23.3)	
	rs1042714	<i>ADRB2</i> Exon 1, Gln27Glu	41	CC (Gln)	95/148	23/17	2.0 (0.9-4.2)	0.68
				CG/GG (GlnGlu)/ (Glu)	187/264	49/37	1.6 (0.9-2.9)	
	rs1042717	<i>ADRB2</i> Exon 1	19	GG	193/251	51/33	1.7 (1.0-3.0)	0.82
				GA/AA	79/142	21/21	1.5 (0.7-3.3)	
	rs1042718	<i>ADRB2</i> Exon 1	15	CC	207/291	54/34	1.9 (1.1-3.4)	0.47
				CA/AA	70/121	16/19	1.4 (0.6-3.1)	

* Early maternal smoking was considered present if the mother smoked at least one cigarette per day at the time of enrolment (2 month's age of a child) and/or smoked at least one cigarette per day in any point of time during pregnancy.

Early onset wheeze was defined as wheeze up to 2 years of age (having transient wheeze defined as ≥ 3 episodes of wheeze between 3 months and 2 years of age, but no episode in the last 12 months at 4 years;

or persistent wheeze defined as ≥ 1 episode of wheeze between 3 months and 2 years of age and ≥ 1 episode in the last 12 months at 4 years).

† Numbers of cases and controls.

‡ Adjusted for sex, mother's age, breast feeding, socioeconomic status, environmental tobacco smoke exposure at 1, 2 and 4 years.

§ Unexposed to tobacco in each genotype category constitute the reference group for each OR. Interaction effects with *TNF* and *ADRB2* SNPs were estimated in dominant genetic models. *GSTP1* SNPs were assessed with genotype-specific model, except for Ala114Val SNP, where dominant model was used because of few subjects with rare homozygote genotype.

Table S4. Odds Ratios for current asthma, late onset wheeze and sensitisation in children followed up to 4 years: effect modification of early maternal smoking exposure* by *TNF*, *GSTP1*, and *ADRB2* gene variants.

Pheno- type	SNP	Gene and Locus	Minor allele frequen cy, %	Genotype	Interaction analysis			
					Unexposed to smoking: Ca / Co†	Exposed to smoking: Ca / Co†	OR (95% CI)‡ for maternal smoking exposure stratified by genotypes§	Intera ction p- value
Current asthma N=136	rs1799964	<i>TNF</i> -1031	21	TT	65/257	17/31	2.2 (1.1-4.2)	0.28
				TC/CC	37/142	7/23	1.2 (0.5-2.9)	
	rs1799724	<i>TNF</i> -857	9	CC	90/347	21/38	2.1 (1.2-3.8)	0.10
				CT/TT	19/73	3/17	0.7 (0.2-2.6)	
	rs1800629	<i>TNF</i> -308	16	GG	66/292	14/41	1.5 (0.8-2.9)	0.32
				GA/AA	39/115	10/11	2.7 (1.1-6.8)	
	rs1800610	<i>TNF</i> Intron 1	9	CC	90/354	21/38	2.2 (1.2-3.9)	0.16
				CT/TT	19/70	4/17	0.9 (0.3-2.9)	
	rs3093664	<i>TNF</i> Intron 3	8	AA	95/356	23/51	1.7 (1.0-2.9)	0.64
				AG/GG	16/63	1/4	1.0 (0.1-9.4)	
	rs762803	<i>GSTP1</i> Intron 4	41	CC	36/157	7/19	1.6 (0.6-4.1)	0.61
				CA	51/196	15/29	2.0 (1.0-4.0)	
	rs1695	<i>GSTP1</i> Exon 5,	33	AA	24/71	2/7	0.8 (0.2-4.3)	0.95
				AA (Ile)	46/190	9/24	1.5 (0.7-3.6)	
	rs749174	<i>GSTP1</i> Intron 5	34	AG (Ile/Val)	53/175	15/27	1.8 (0.9-3.7)	0.84
				GG (Val)	10/51	1/3	1.7 (0.2-18.0)	
	rs1138272	<i>GSTP1</i> Exon 6, Ala114Val	9	CC	40/193	8/26	1.5 (0.6-3.5)	0.86
				CT	61/174	14/25	1.6 (0.8-3.3)	
	rs1871042	<i>GSTP1</i> Intron 6	33	TT	10/55	2/4	2.8 (0.4-17.1)	0.85
				CC (Ala)	79/339	19/43	1.9 (1.0-3.4)	
	rs4891	<i>GSTP1</i> Exon 7	30	CT/TT (AlaVal)/ (Val)	29/58	5/6	1.7 (0.5-5.9)	0.79
				CC	44/198	10/26	1.7 (0.8-3.8)	
Late onset wheeze N=131	rs1042714	<i>ADRB2</i> Exon 1, Gln27Glu	41	CT	54/170	13/25	1.6 (0.8-3.4)	0.71
				TT	12/54	2/3	3.0 (0.5-20.0)	
	rs1042717	<i>ADRB2</i> Exon 1	19	TT	50/205	11/29	1.6 (0.7-3.3)	0.53
				TC	44/148	11/22	1.7 (0.8-3.7)	
	rs1042718	<i>ADRB2</i> Exon 1	15	CC	10/49	2/3	3.3 (0.5-22.2)	0.24
				CG/GG (GlnGlu)/ (Glu)	40/150	9/17	2.0 (0.8-4.8)	
	rs1799964	<i>TNF</i> -1031	21	GG	70/268	16/38	1.6 (0.8-3.1)	0.44
				GA/AA	72/254	18/33	1.9 (1.0-3.6)	
	rs1799724	<i>TNF</i> -857	9	GA/AA	34/144	7/22	1.3 (0.5-3.4)	0.66
				CC	79/295	19/34	2.1 (1.1-3.9)	
Late onset wheeze N=131	rs1800629	<i>TNF</i> -308	16	CA/AA	30/122	5/20	1.0 (0.4-2.9)	0.88
				GG	62/257	10/31	1.3 (0.6-2.9)	
	rs1799724	<i>TNF</i> -857	9	TC/CC	45/142	6/23	0.8 (0.3-2.1)	0.66
				CC	97/347	12/38	1.1 (0.6-2.2)	
Late onset wheeze N=131	rs1800629	<i>TNF</i> -308	16	CT/TT	14/73	5/17	1.5 (0.5-4.8)	0.88
				GG	76/292	11/41	1.0 (0.5-2.1)	
	rs1800629	<i>TNF</i> -308	16	GA/AA	27/115	3/11	1.2 (0.3-4.5)	0.88
				GA/AA	27/115	3/11	1.2 (0.3-4.5)	

Pheno- type	SNP	Gene and Locus	Minor allele frequen cy, %	Genotype	Interaction analysis			
					Unexposed to smoking: Ca / Co†	Exposed to smoking: Ca / Co†	OR (95% CI)‡ for maternal smoking exposure stratified by genotypes§	Intera ction p- value
Late onset wheeze N=131	rs1800610	<i>TNF</i>	9	CC	97/354	12/38	1.2 (0.6-2.3)	0.98
		Intron 1		CT/TT	14/70	4/17	1.2 (0.3-4.0)	
	rs3093664	<i>TNF</i>	8	AA	93/356	14/51	1.1 (0.6-2.0)	0.57
		Intron 3		AG/GG	17/63	2/4	1.9 (0.3-11.0)	
	rs762803	<i>GSTP1</i>	41	CC	47/157	8/19	1.4 (0.6-3.4)	0.48
		Intron 4		CA/AA	65/267	8/36	0.9 (0.4-2.1)	
	rs1695	<i>GSTP1</i>	33	AA (Ile)	57/190	8/24	1.1 (0.5-2.6)	0.81
		Exon 5, Ile105Val		AG/GG (Ile/Val)/ (Val)	53/226	9/30	1.3 (0.6-2.9)	
	rs749174	<i>GSTP1</i>	34	CC	50/193	6/26	0.9 (0.3-2.3)	0.67
		Intron 5		CT/TT	61/229	9/29	1.2 (0.5-2.6)	
	rs1138272	<i>GSTP1</i>	9	CC (Ala)	90/339	14/43	1.2 (0.6-2.3)	0.75
		Exon 6, Ala114Val		CT/TT (AlaVal)/ (Val)	21/58	2/6	0.9 (0.2-4.9)	
	rs1871042	<i>GSTP1</i>	33	CC	57/198	9/26	1.2 (0.5-2.7)	0.96
		Intron 6		CT/TT	55/224	8/28	1.2 (0.5-2.7)	
	rs4891	<i>GSTP1</i>	30	TT	59/205	9/29	1.1 (0.5-2.4)	0.98
		Exon 7		TC/CC	52/197	7/25	1.1 (0.4-2.6)	
rs1042714	<i>ADRB2</i>	41	CC (Gln)	41/150	9/17	1.9 (0.8-4.7)	0.15	
	Exon 1, Gln27Glu		CG/GG (GlnGlu)/ (Glu)	70/268	8/38	0.8 (0.4-1.8)		
rs1042717	<i>ADRB2</i>	19	GG	67/254	6/33	0.7 (0.3-1.7)	0.16	
	Exon 1		GA/AA	40/144	10/22	1.6 (0.7-3.7)		
rs1042718	<i>ADRB2</i>	15	CC	78/295	8/34	0.9 (0.4-2.0)	0.33	
	Exon 1		CA/AA	34/122	9/20	1.6 (0.7-3.9)		
Sensiti sation N=266	rs1799964	<i>TNF</i> -1031	21	TT	127/196	24/22	1.7 (0.9-3.1)	0.84
	rs1799724	<i>TNF</i> -857	9	TC/CC	81/108	16/14	1.5 (0.7-3.3)	0.69
				CC	186/266	30/26	1.7 (0.9-2.9)	
	rs1800629	<i>TNF</i> -308	16	CT/TT	36/52	10/11	1.3 (0.5-3.4)	0.52
				GG	135/235	28/28	1.7 (1.0-3.1)	
	rs1800610	<i>TNF</i>	9	GA/AA	75/72	10/8	1.2 (0.4-3.2)	0.63
				CC	185/270	30/26	1.7 (1.0-2.9)	
	rs3093664	<i>TNF</i>	8	CT/TT	36/51	10/11	1.3 (0.5-3.4)	0.36
				AA	190/266	35/35	1.4 (0.8-2.3)	
	rs762803	<i>GSTP1</i>	41	AG/GG	32/51	4/2	3.2 (0.6-18.4)	0.71
				CC	77/121	14/11	2.0 (0.9-4.6)	
	rs1695	<i>GSTP1</i>	33	CA	103/147	19/20	1.4 (0.7-2.7)	0.37
				AA	44/52	6/6	1.2 (0.4-3.9)	
	rs749174	<i>GSTP1</i>	34	AA (Ile)	95/146	17/15	1.7 (0.8-3.7)	0.38
				AG (Ile/Val)	104/128	18/20	1.1 (0.6-2.2)	
	rs749174	<i>GSTP1</i>	34	GG (Val)	22/41	4/2	3.7 (0.6-22.0)	0.38
CC				96/143	17/15	1.7 (0.8-3.5)		
			CT	104/131	16/19	1.1 (0.5-2.2)		
			TT	24/45	5/3	3.1 (0.7-14.2)		

Pheno- type	SNP	Gene and Locus	Minor allele frequen- cy, %	Genotype	Interaction analysis			
					Unexposed to smoking: Ca / Co†	Exposed to smoking: Ca / Co†	OR (95% CI)‡ for maternal smoking exposure stratified by genotypes§	Intera- ction p- value
Sensiti- sation N=266	rs1138272	<i>GSTP1</i>	9	CC (Ala)	176/255	31/30	1.5 (0.9-2.6)	1.00
		Exon 6, Ala114Val		CT/TT (AlaVal)/ (Val)	36/43	5/4	1.5 (0.4-6.0)	
	rs1871042	<i>GSTP1</i> Intron 6	33	CC	97/148	19/15	1.9 (0.9-4.0)	0.11
				CT	104/126	15/20	0.9 (0.4-1.9)	
				TT	23/45	5/2	4.9 (0.9-27.2)	
	rs4891	<i>GSTP1</i> Exon 7	30	TT	102/155	19/18	1.6 (0.8-3.2)	0.23
				TC	89/111	14/17	1.0 (0.5-2.2)	
				CC	21/40	5/2	4.8 (0.9-26.7)	
	rs1042714	<i>ADRB2</i> Exon 1, Gln27Glu	41	CC (Gln)	91/106	14/10	1.6 (0.7-3.8)	0.93
				CG/GG (GlnGlu)/ (Glu)	130/210	26/27	1.6 (0.9-2.8)	
	rs1042717	<i>ADRB2</i> Exon 1	19	GG	129/197	27/23	1.8 (1.0-3.3)	0.48
	rs1042718	<i>ADRB2</i> Exon 1	15	GA/AA	79/106	13/14	1.2 (0.6-2.8)	0.47
				CC	152/225	27/24	1.7 (0.9-3.0)	
				CA/AA	68/91	11/13	1.1 (0.5-2.7)	

* Early maternal smoking was considered present if the mother smoked at least one cigarette per day at the time of enrolment (2 month's age of a child) and/or smoked at least one cigarette per day in any point of time during pregnancy.

Current asthma was defined as reported physician-diagnosed asthma up to 4 years of age and one or more episodes of wheezing in the preceding 12 months at 4 years.

Late-onset wheeze was defined as no episode of wheeze between 3 months and 2 years of age, but ≥ 1 episode in the last 12 months at 4 years.

Allergic sensitisation was defined as serum IgE antibody level ≥ 0.35 kU_A/l.

† Numbers of cases and controls.

‡ Crude models.

§ Unexposed to tobacco in each genotype category constitute the reference group for each OR.

Interaction effects with *TNF* and *ADRB2* SNPs were estimated in dominant genetic models. *GSTP1* SNPs were assessed with genotype-specific model, except for Ala114Val SNP and late-onset wheeze estimates, where dominant model was used because of few subjects with rare homozygote genotype.

Table S5. Odds Ratios for transient and persistent wheeze in children followed up to 4 years: effect modification of early maternal smoking exposure* by *TNF* and *GSTP1* gene variants.

Pheno- type	SNP	Gene and Locus	Minor allele frequen cy, %	Genotype	Interaction analysis			
					Unexposed to smoking: Ca / Co†	Exposed to smoking: Ca / Co†	OR (95% CI)‡ for maternal smoking exposure stratified by genotypes§	Intera ction p- value
Transient wheeze N=155	rs1799724	TNF -857	9	CC	109/347	28/38	2.3 (1.4-4.0)	0.02
				CT/TT	14/73	1/17	0.3 (0.04-2.5)	
	rs1800610	TNF Intron 1	9	CC	107/354	27/38	2.4 (1.4-4.0)	0.01
				CT/TT	16/70	1/17	0.3 (0.03-2.1)	
	rs3093664	TNF Intron 3	8	AA	108/356	22/51	1.4 (0.8-2.5)	0.04
				AG/GG	18/63	7/4	6.1 (1.6-23.3)	
	rs1695	GSTP1 Ile105Val	33	AA (Ile)	58/190	9/24	1.2 (0.5-2.8)	0.06
				AG	51/175	12/27	1.5 (0.7-3.2)	
				(IleVal) GG (Val)	14/51	7/3	8.5 (1.9-37.2)	
	rs749174	GSTP1 Intron 5	34	CC	57/193	8/26	1.0 (0.4-2.4)	0.05
				CT	54/174	12/25	1.5 (0.7-3.3)	
				TT	13/55	7/4	7.4 (1.9-29.1)	
	rs1871042	GSTP1 Intron 6	33	CC	61/198	9/26	1.1 (0.5-2.5)	0.02
				CT	52/170	12/25	1.6 (0.7-3.3)	
				TT	12/54	7/3	10.5 (2.4-46.6)	
Persistent wheeze N=211	rs1799724	TNF-857	9	CC	133/347	36/38	2.5 (1.5-4.1)	0.02
				CT/TT	32/73	5/17	0.7 (0.2-2.0)	
	rs1800610	TNF Intron 1	9	CC	132/354	36/38	2.5 (1.5-4.2)	0.03
				CT/TT	32/70	6/17	0.8 (0.3-2.1)	
	rs3093664	TNF Intron 3	8	AA	146/356	35/51	1.7 (1.0-2.7)	0.06
				AG/GG	21/63	8/4	6.0 (1.6-22.0)	

* Early maternal smoking was considered present if the mother smoked at least one cigarette per day at the time of enrolment (2 month's age of a child) and/or smoked at least one cigarette per day in any point of time during pregnancy.

Transient wheeze was defined as ≥ 3 episodes of wheeze between 3 months and 2 years of age, but no episode in the last 12 months at 4 years.

Persistent wheeze was defined as ≥ 1 episode of wheeze between 3 months and 2 years of age and ≥ 1 episode in the last 12 months at 4 years.

† Numbers of cases and controls.

‡ Crude models.

§ Unexposed to tobacco in each genotype category constitute the reference group for each OR.

Table S6. Odds ratios for early onset wheeze in children followed up to 4 years in relation to extent of maternal smoking during pregnancy (non smoking, moderate: 1-9 cigarettes per day, and high: >9 cigarettes per day*), stratified by genetic variation in the *TNF* -857 (rs1799724) SNP.

Number of cigarettes smoked by mother per day during pregnancy*	<i>TNF</i> -857 CC (83.25%)†		<i>TNF</i> -857 CT/TT (16.75%)†	
	Ca/Co‡	OR (95% CI)§	Ca/Co‡	OR (95% CI)§
0	246/349	1.0	46/73	1.0
1-9	19/19	1.4 (0.7-2.7)	2/7	0.5 (0.1-2.3)
> 9	41/17	3.4 (1.9-6.2)	4/10	0.6 (0.2-2.1)

Early onset wheeze was defined as wheeze up to 2 years of age (having transient wheeze defined as ≥ 3 episodes of wheeze between 3 months and 2 years of age, but no episode in the last 12 months at 4 years; or persistent wheeze defined as ≥ 1 episode of wheeze between 3 months and 2 years of age and ≥ 1 episode in the last 12 months at 4 years).

* Corresponding to the highest number of cigarettes usually smoked per day by mother during any of the pregnancy trimesters.

† Frequency of genotype in studied population.

‡ Numbers of cases and controls.

§ ORs are estimated in crude models.

Figure S1. Block structure and correlation of the SNPs in the *TNF* gene (A), *GSTP1* gene (B), and *ADRB2* gene (C). The numbers in each box correspond to the pairwise correlation coefficient r^2 between respective SNPs. Defining the block structure was based on the linkage disequilibrium coefficient D' using Haploview (Solid spine of LD).

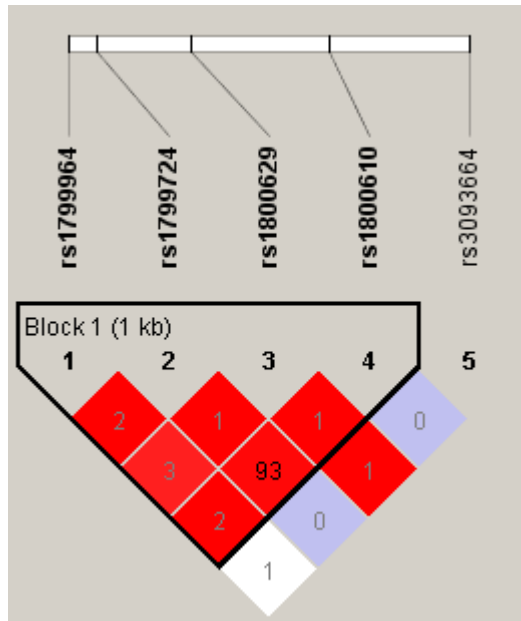


Figure S1A

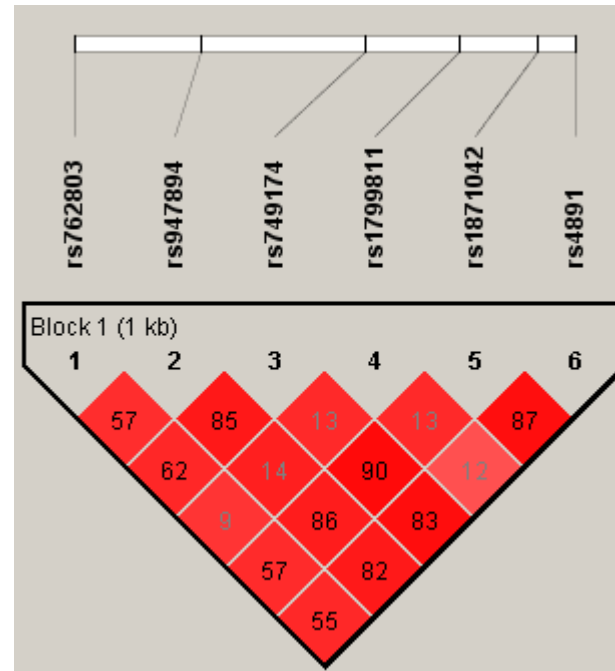


Figure S1B

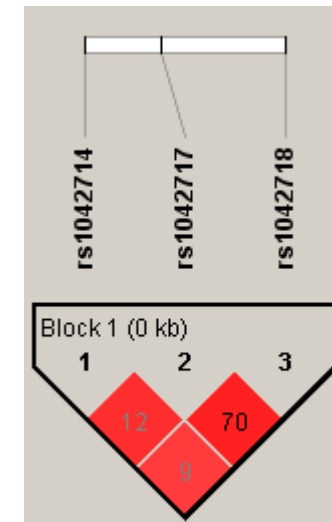


Figure S1C