

ORIGINAL ARTICLE

EPIDEMIOLOGY AND GENETICS

Interaction of a 17q12 variant with both fetal and infant smoke exposure in the development of childhood asthma-like symptoms

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Abstract

Background: Gene variants on chromosome 17q12-21 are associated with an increased risk of childhood-onset asthma, a risk known to be modified by environmental tobacco smoke (ETS).

Objectives: To assess whether the association of rs2305480 on chromosome 17q12 in the *GSDML* gene with asthma-like symptoms in the first 4 years of life is modified by smoke exposure during fetal and early postnatal life.

Methods: We used data from two independent prospective cohort studies from fetal life onwards in the Netherlands. We genotyped rs2305480 and assessed maternal smoking during pregnancy and ETS exposure at the age of 2. Asthma-like symptoms, defined as any reported wheezing, shortness of breath or dry nocturnal cough, were reported by parents when the children were 1, 2, 3, and 4 years. Analyses were based on a total group of 4461 Caucasian children.

Results: The G risk-allele of rs2305480 was associated with asthma-like symptoms [overall odds ratio 1.17 (1.11, 1.24), 2.66E-9]. The effect of rs2305480 on asthma-like symptoms was stronger among children who were exposed to smoke during fetal life (*P*-interaction = 0.04). Smoke exposure in early postnatal life was also associated with an increased effect of the 17q12 single nucleotide polymorphism (SNP) on asthma-like symptoms (*P*-interaction = 5.06E-4). These associations were consistent in both cohorts.

Conclusion: A 17q12 variant, rs2305480, was associated with asthma-like symptoms in preschool children, and this association was modified by smoke exposure already during fetal life, and in infancy. Further investigation regarding SNPs in linkage disequilibrium with rs2305480 in relation to pathophysiological pathways is needed.

Genome-wide association studies (GWAS) identified single nucleotide polymorphisms (SNPs) on chromosome 17q12-21 that are associated with childhood asthma (1–3). The associa-

tions have been replicated in independent populations (1–9). Rs2305480 in gasdermin-like (*GSDML*) gene on 17q12 showed the strongest association with childhood asthma (3)

and was associated with transcript levels of ORM1-like 3 (*ORMDL3*) (1) and GSDML (10). Gene–environment interactions contribute to the development of asthma (2). An interaction between 17q12-21 variants, including rs2305480, and environmental tobacco smoke (ETS) exposure in the first years of life with asthma phenotypes has been published previously (8, 9). Whether the effect of rs2305480 variants on asthma-like symptoms is modified by fetal smoke exposure is unknown (2). Exploration of a possible interaction between rs2305480, fetal smoke exposure, and ETS exposure in infancy may identify specific early critical time periods of increased susceptibility (2, 9). In this study, we examined in two independent prospective birth cohort studies whether the association between rs2305480 and asthma-like symptoms was modified by smoke exposure already during fetal life and by ETS exposure in infancy.

Methods

We used data from two independent prospective birth cohort studies from fetal life onwards in the Netherlands. In the Generation R Study (11), recruitment by midwives and obstetricians from the Rotterdam area took place between July 2001 and January 2006, and for the 'Prevention and Incidence of Asthma and Mite Allergy' (PIAMA) study (12), recruitment took place in 1996–1997 through prenatal clinics in Groningen, Rotterdam, and Utrecht. Both studies have been approved by the local Medical Ethics Committees. Analyses were restricted to singleton live-births with Caucasian ethnicity and subjects from whom DNA was available for genotyping (13). Caucasian ethnicity was defined as having principal components within four SD values of the CEU cluster of HapMap (14). We genotyped rs2305480 in the *GSDML* gene. Causal SNP(s) may lie elsewhere in the block of linkage disequilibrium (LD) (2). In the Generation R Study, genotyping was performed using Illumina 610k Quad arrays (San Diego, CA, USA) and in the PIAMA study by KBioscience KASP SNP genotyping technology (Hoddesdon, Herts, England). The genotype frequencies of rs2305480 were 22.2% (AA), 48.3% (GA), and 29.5% (GG) (Hardy–Weinberg $P = 0.16$) in the Generation R Study and in PIAMA 19.2% (AA), 49.4% (GA), and 31.4% (GG) (Hardy–Weinberg $P = 0.56$).

Respiratory symptoms were assessed by questionnaires at the age of 1, 2, 3, and 4. Questions were taken from the International Study of Asthma and Allergies in Childhood (ISAAC) (15). For this analysis, we defined asthma-like symptoms as any reported wheezing, shortness of breath or dry nocturnal cough without a cold in a given year (16). Information about fetal smoke exposure and ETS exposure in infancy was assessed by postal questionnaires during pregnancy and at the age of 2, asking the mothers whether they had smoked during pregnancy and whether people smoked occasionally in the house, respectively (12, 17). In this analysis, first trimester smokers and mothers who continued smoking during pregnancy were combined.

Analyses were based on a total group of 4461 subjects. We evaluated the strength of association (SNP effect)

assuming an additive genetic model. The associations of rs2305480 with asthma-like symptoms in children at the age of 1, 2, 3, and 4 and the overall effects at all ages were analyzed using multiple imputation-based generalized estimating equation (MI-GEE) models (18, 19). To test for effect modification, we calculated terms for interactions between rs2305480 and smoke exposure status in the MI-GEE model. All models were adjusted for maternal age, education and parity, children's sex, gestational age, birth-

Table 1 Baseline characteristics of mothers and children ($n = 4461$)

	Generation R ($n = 2438$)	PIAMA ($n = 2023$)
Maternal characteristics		
Age, years	31.4 (4.2)	30.7 (3.8)
Highest completed education (%)		
Primary or secondary	37.6 (916)	62.2 (1259)
Higher	62.4 (1522)	37.8 (764)
Child characteristics		
Female (%)	49.0 (1195)	48.7 (986)
Gestational age at birth (weeks)	40.3 (37.4–42.1)	40.1 (37.1–41.9)
Birthweight (g)	3534 (520)	3531 (530)
Familial history of asthma or atopy (%)		
No	47.3 (1153)	43.3 (875)
Yes	52.7 (1285)	56.7 (1148)
Breastfed (%)		
No	11.2 (274)	15.6 (315)
Yes	88.8 (2164)	84.4 (1708)
Daycare attendance 1st year (%)		
No	40.2 (979)	75.2 (1521)
Yes	59.8 (1459)	24.8 (502)
Pets in house (%)		
No	56.2 (1370)	48.8 (987)
Yes	43.8 (1068)	51.2 (1036)
Smoke exposure (%)		
No smoke	50.2 (1225)	55.6 (1125)
Fetal smoke only	9.1 (222)	4.6 (93)
Infant smoke only	4.8 (117)	22.5 (455)
Fetal and infant smoke	5.8 (142)	15.2 (308)
Missing	30.0 (732)	2.1 (42)
Asthma-like symptoms (%)		
Year 1*	50.1 (1222)	32.4 (656)
Year 2*	41.3 (1006)	27.1 (548)
Year 3	33.8 (825)	34.5 (698)
Year 4	34.3 (836)	33.1 (671)

Values are means (standard deviation), medians (5–95th percentiles), or percentages (absolute numbers). Pooled estimates were calculated of the ten multiple imputations generated independent datasets.

*Shortness of breath question not available in 'Prevention and Incidence of Asthma and Mite Allergy' (PIAMA) at age of 1 and 2 years.

weight, familial history of asthma and allergy, breastfeeding, daycare attendance, and pet keeping, based on the significance of their associations with asthma-like symptoms ($P < 0.05$), or a change in effect estimate of $>10\%$. Missing values in covariates were low (highest 9.6%). To handle missing values in binary repeated outcomes (18, 19) and covariates, but not for the determinants 'fetal smoke exposure' and 'ETS exposure in infancy', multiple imputations were used for all analyses. Ten independent datasets were generated, and pooled estimates were calculated. Imputations were based on the relationships between all potential confounders. No differences in results were observed between analyses with imputed data or complete cases (data not shown). Statistical analyses were performed using SAS 9.2 (SAS Institute, Cary, NC, USA). Combined effect estimates and heterogeneity between cohorts were calculated using fixed-effect meta-analyses in R Version 2.8.1 (library rmeta; The R foundation for Statistical Computing).

Results

Descriptive baseline characteristics of mothers and children were comparable in both cohorts, with the exception of education levels of the mother and daycare attendance, which were higher in the Generation R Study (Table 1). Early-life smoke exposure was more frequent in the PIAMA cohort.

The prevalence of asthma-like symptoms according to fetal and infant smoke exposure categories varied between 25 and over 50% and was higher in children who were exposed compared to those who were not exposed to smoke (Table 2).

Overall, the G risk-allele of rs2305480 was associated with asthma-like symptoms from birth until 4 years of age in both the Generation R Study and PIAMA [overall combined odds ratio (OR) 1.17 95% confidence interval (1.11, 1.24), $p = 2.66E-9$] (Table 3). The associations were significant for each year separately for age 2–4 years, but not for the first year. The strongest associations were seen in children aged 3 and 4 years with both the risk-allele and exposure to ETS in fetal and early life (Tables 4 and 5).

The associations between rs2305480 and asthma-like symptoms were modified by fetal smoke exposure (combined P -interaction = 0.04) and ETS exposure in infancy (combined P -interaction = $5.06E-4$) (Fig. 1). We did not find an overall SNP effect in the group with both fetal and early-life smoke exposure in the Generation R Study and PIAMA study. In the Generation R Study, complete data on fetal and early-life smoke exposure were missing in 30%, and in PIAMA 2.1%. A sensitivity analysis for missing data in Generation R showed that the effect of rs2305480 on asthma-like symptoms was comparable in children of whom complete smoking data were not available and in children that were not exposed [OR 1.19 (.95–1.47) and OR 1.10 (1.01–1.19),

Table 2 Prevalence of asthma-like symptoms according to fetal and infant smoke exposure

	Generation R					PIAMA				
	No smoke ($n = 1225$)	Fetal only ($n = 222$)	Infant only ($n = 117$)	Fetal and infant ($n = 142$)	Missing ($n = 732$)	No smoke ($n = 1125$)	Fetal only ($n = 93$)	Infant only ($n = 445$)	Fetal and infant ($n = 308$)	Missing ($n = 42$)
Year 1*	49.1 (602)	53.8 (120)	41.8 (49)	51.1 (73)	51.7 (379)	33.6 (378)	45.2 (42)	27.0 (123)	32.5 (100)	33.3 (14)
Year 2*	38.5 (472)	44.9 (100)	52.0 (61)	42.3 (60)	42.9 (314)	26.3 (296)	35.5 (33)	25.9 (118)	27.6 (85)	35.7 (15)
Year 3	31.8 (390)	36.6 (81)	34.4 (40)	34.4 (49)	36.2 (265)	33.4 (376)	49.5 (46)	32.7 (149)	35.1 (108)	47.6 (20)
Year 4	32.5 (398)	37.1 (82)	42.1 (49)	33.1 (47)	35.4 (259)	32.2 (363)	44.1 (41)	29.7 (135)	38.0 (117)	35.7 (15)
Overall	38.0 (465)	43.1 (96)	42.6 (50)	40.2 (57)	41.6 (304)	31.4 (353)	43.6 (41)	28.8 (131)	33.3 (103)	38.1 (16)

Values are percentages N /total of stratum (N = number of asthma-like symptom cases). Pooled estimates were calculated of the ten multiple imputations generated independent datasets.

*Shortness of breath question not available in 'Prevention and Incidence of Asthma and Mite Allergy' (PIAMA) at age of 1 and 2 years.

Table 3 Association between rs2305480 and asthma-like symptoms

Asthma-like symptoms at age	Generation R ($n = 2438$) OR (95% CI) for asthma-like symptoms*	PIAMA ($n = 2023$) OR (95% CI) for asthma-like symptoms*	Combined ($n = 4461$) OR (95% CI) for asthma-like symptoms*	Hetero- geneity, P
Year 1†	1.05 (0.92–1.19)	1.10 (0.96–1.27)	1.07 (0.98–1.18)	0.595
Year 2†	1.17 (1.02–1.34)	1.13 (0.98–1.31)	1.15 (1.05–1.27)	0.760
Year 3	1.25 (1.07–1.46)	1.31 (1.15–1.50)	1.28 (1.16–1.42)	0.658
Year 4	1.17 (1.00–1.37)	1.20 (1.05–1.38)	1.19 (1.07–1.32)	0.779
Overall	1.15 (1.06–1.26)	1.19 (1.11–1.27)	1.17 (1.11–1.24)	0.554

*Overall odds ratios (OR) and 95% confidence intervals (CI) for asthma-like symptoms are given for the risk-allele G in the total sample.

†Shortness of breath question not available in 'Prevention and Incidence of Asthma and Mite Allergy' (PIAMA). ORs were given (allowing for a time trend) for each year of age separately and for the overall effect. Models are adjusted for gender, maternal age, familial atopy history, breastfeeding, and daycare attendance.

Table 4 Association between rs2305480, fetal smoke exposure, and asthma-like symptoms

Asthma-like symptoms at age	Generation R (E- n = 1696 : E+ n = 540)		PIAMA (E- n = 1600 : E+ n = 411)		Combined (E- n = 3296 : E+ n = 951)	
	OR (95% CI) for asthma-like symptoms*		OR (95% CI) for asthma-like symptoms*		OR (95% CI) for asthma-like symptoms*	
	E-	E+	E-	E+	E-	Hetero- genity, P
Year 1 [†]	1.01 (0.87–1.18)	1.05 (0.77–1.42)	1.14 (0.98–1.33)	0.94 (0.69–1.27)	1.07 (0.97–1.19)	0.278
Year 2 [†]	1.15 (0.99–1.35)	1.06 (0.76–1.48)	1.09 (0.93–1.28)	1.21 (0.89–1.67)	1.12 (1.01–1.26)	0.626
Year 3	1.19 (1.00–1.41)	1.33 (0.97–1.82)	1.28 (1.10–1.49)	1.38 (1.03–1.86)	1.24 (1.11–1.39)	0.531
Year 4	1.12 (0.95–1.32)	1.26 (0.86–1.84)	1.16 (0.99–1.35)	1.32 (0.98–1.79)	1.14 (1.02–1.28)	0.800
Overall	1.11 (1.02–1.22)	1.16 (0.93–1.44)	1.17 (1.08–1.27)	1.21 (1.03–1.41)	1.15 (1.08–1.21)	0.390

*Overall odds ratios (OR) and 95% confidence intervals (CI) for asthma-like symptoms are given for the risk-allele G in all children where fetal smoke exposure information was available (E- not exposed; E+ exposed children).

[†]Shortness of breath question not available in 'Prevention and Incidence of Asthma and Mite Allergy' (PIAMA). ORs were given (allowing for a time trend) for each year of age separately and for the overall effect. Models are adjusted for gender, maternal age, familial atopy history, breastfeeding, and daycare attendance.

Table 5 Association between rs2305480, infant smoke exposure, and asthma-like symptoms

Asthma-like symptoms at age	Generation R (E- n = 1582 : E+ n = 284)		PIAMA (E- n = 1225 : E+ n = 768)		Combined (E- n = 2807 : E+ n = 1052)	
	OR (95% CI) for asthma-like symptoms*		OR (95% CI) for asthma-like symptoms*		OR (95% CI) for asthma-like symptoms*	
	E-	E+	E-	E+	E-	Hetero- genity, P
Year 1 [†]	1.06 (0.92–1.22)	1.06 (0.72–1.57)	1.10 (0.93–1.31)	1.10 (0.87–1.38)	1.08 (0.97–1.20)	0.719
Year 2 [†]	1.22 (1.06–1.40)	0.98 (0.69–1.40)	1.01 (0.84–1.21)	1.36 (1.07–1.72)	1.13 (1.01–1.27)	0.111
Year 3	1.23 (1.05–1.44)	1.91 (1.25–2.92)	1.26 (1.06–1.49)	1.39 (1.11–1.74)	1.24 (1.10–1.39)	0.847
Year 4	1.18 (1.01–1.37)	1.27 (0.86–1.87)	1.08 (0.91–1.28)	1.46 (1.16–1.83)	1.13 (1.01–1.27)	0.454
Overall	1.16 (1.08–1.25)	1.23 (1.01–1.49)	1.11 (1.02–1.22)	1.32 (1.18–1.48)	1.14 (1.08–1.21)	0.466

*Overall odds ratios (OR) and 95% confidence intervals (CI) for asthma-like symptoms are given for the risk-allele G in all children where infant smoke exposure information was available (E- not exposed; E+ exposed children).

[†]Shortness of breath question not available in 'Prevention and Incidence of Asthma and Mite Allergy' (PIAMA). ORs were given (allowing for a time trend) for each year of age separately and for the overall effect. Models are adjusted for gender, maternal age, familial atopy history, breastfeeding, and daycare attendance.

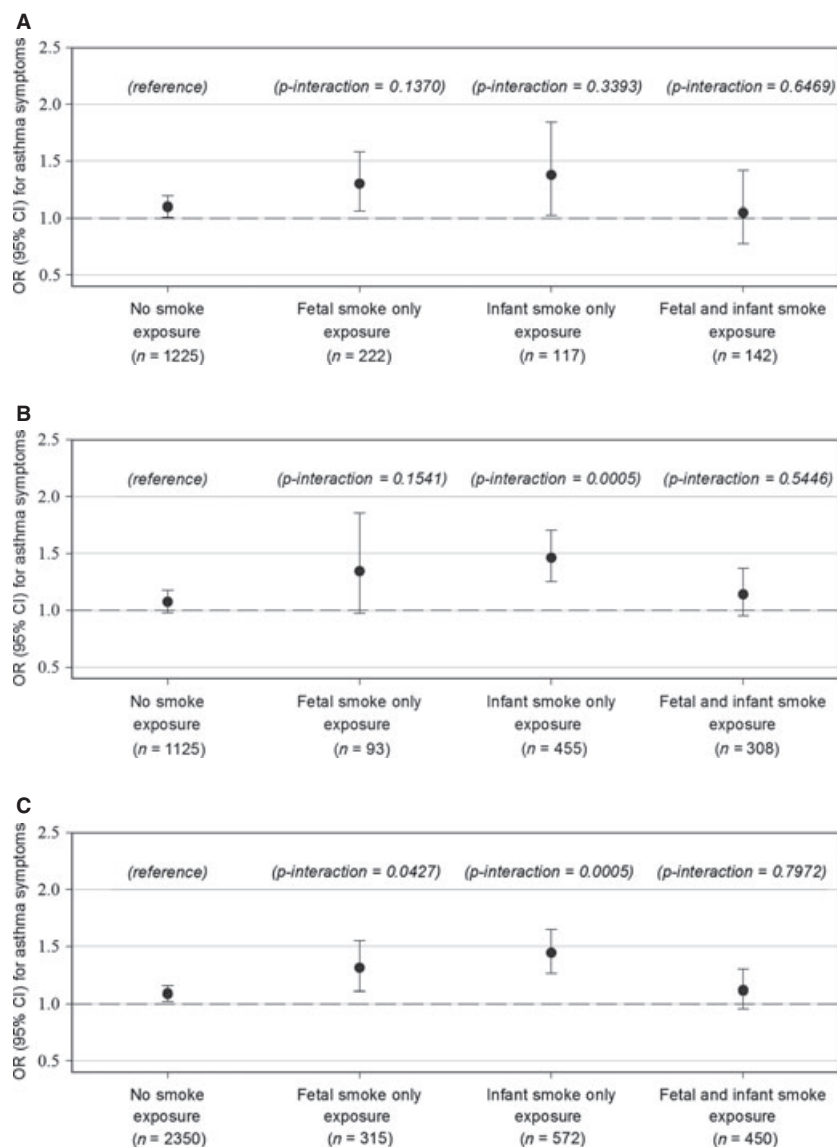


Figure 1 Overall association between rs2305480 and asthma-like symptoms, according to fetal and infant smoke exposure. Overall odds ratios (OR) and 95% confidence intervals (CI) for asthma-like symptoms are given for the risk-allele G in (A) the Generation R Study, (B) 'Prevention and Incidence of Asthma and Mite Allergy' (PIAMA), and (C) combined. Models are adjusted for gender, maternal age, familial atopy history, breastfeeding, and daycare

attendance. For this analysis, it was required to have information available for both fetal and infant smoke exposure. The following strata were depicted: No smoke (the Generation R Study $n = 1225$, PIAMA $n = 1125$); fetal smoke only (the Generation R Study $n = 222$, PIAMA $n = 93$); infant smoke only (the Generation R Study $n = 117$, PIAMA $n = 455$); fetal and infant smoke exposure (the Generation R Study $n = 142$, PIAMA $n = 308$).

respectively, P -interaction = 0.52]. The directions of the SNP effects were consistent between the Generation R Study and PIAMA for all analyses (heterogeneity P -values > 0.05).

Discussion

We found that a common 17q12-21 variant, rs2305480, was associated with asthma-like symptoms in preschool children and for the first time demonstrated that smoke exposure modified this effect of rs2305480 already during fetal life.

Previous studies have shown associations between 17q12-21 variants and early-onset asthma (1–9). We replicated these findings for asthma-like symptoms in preschool children in two independent birth cohorts. The increased risk of asthma conferred by rs2305480 was enhanced by early childhood ETS exposure (8, 9), which was also observed in the present study. To our knowledge, there are no reports on the interaction between 17q12-21 variants and fetal smoke exposure on any asthma outcome. We found a modifying effect of fetal smoke exposure on the association between rs2305480 and

asthma-like symptoms. We did not find an association for the first year of life, which may partly be explained by the specific, transient wheezing phenotype that has its highest prevalence at this age and relates to viral infection rather than asthma (20). In children with 17q21 risk genotypes and early-life ETS exposure, associations between infection and asthma were further enhanced (21).

Fetal smoke exposure affects future respiratory health by mechanisms that may include interference with fetal overall growth and lung growth and reduction of fetal breathing movements (22). Maternal smoking during pregnancy decreases expression of genes that are involved in lung development in neonatal mice and increases airway remodeling and hyper-responsiveness in the offspring (23, 24). Evidence of epigenetic interactions with *in utero* smoke exposure in humans is emerging (25, 26), but has not yet been described for *GSDML*. A common disease allele in 17q12-21 was linked to changes in insulator protein CTCF binding and nucleosome occupancy leading to altered domain-wide cis-regulation, and cis-regulatory haplotypes were strongly associated with asthma (27). Previous studies showed that *GSDML* is involved in epithelial barrier function in mice (28, 29). The mechanisms by which ETS affects respiratory health are complex and may involve gene-ETS interactions (26, 30). Indeed, airway inflammation may result from a genetically compromised barrier function of the airway mucosa, allowing airborne allergens to penetrate the mucosa (31).

There are some methodological issues that could have influenced our findings. First, parent-reported asthma-like symptoms were the outcome in our study, and asking for symptoms that occurred in the previous year may not produce accurate results. However, in preschool children, a diagnosis of asthma is based on symptoms (32). This method of assessing symptoms and exposures is widely accepted in epidemiological asthma studies, and we used well-validated questionnaires (15, 16). We were not able to assess the association of rs2305480 and its interaction with fetal and infant smoke exposure with specific childhood asthma phenotypes, including transient, intermediate, late onset, persistent or other wheezing phenotypes (33, 34), owing to the availability of data in the Generation R Study until the age of 4 years only. Follow-up studies at older ages, which include more detailed assessments of childhood asthma phenotypes, are needed. Although assessing smoke exposure by questionnaires is valid, misclassification may occur because of underreporting leading to bias toward the null (35, 36). We addressed this in a previous study, which showed good agreement between the PIAMA questions after ETS exposure and actual measurements of nicotine in indoor air (37). Misclassification may still differ between symptomatic and nonsymptomatic children. The impact of this cannot be measured, but differential misclassification is unlikely as we used data of two prospective birth cohorts. Assessment of smoke exposure in early life could have introduced misclassification, because this was measured at a single point in time, at the age of 2, while variation in levels and duration of exposure could have changed over time. This could not be examined

as smoking was not assessed annually. Baseline demographic characteristics including social status, daycare attendance, and smoke exposure prevalence differed between the two cohorts, but were controlled for in the analysis. Heterogeneity tests showed that there existed no different effects between the two cohorts. In general, the previously shown effect estimates for the associations of common genetic variants with doctor's diagnosis of childhood asthma were small with odds ratios ranging from 1.09 to 1.32 (3). In our study, we found a SNP effect of OR 1.09 overall in unexposed children, 1.31 in the fetal only-exposed group, and 1.44 in the infant only-exposed group. It was puzzling to find a smaller SNP effect in the group with both pre- and postnatal smoke exposure than with either pre- or postnatal separate exposures. We think that this could result from different mechanisms. Heavy smoking parents may underreport respiratory symptoms of their children (38), and 'continuation of parents' smoking after pregnancy might be more likely if children have no respiratory symptoms. Hence, underestimation of symptoms and/or reverse causation might have specifically affected this subgroup. We genotyped rs2305480 in the *GSDML* gene, but causal SNP(s) may lie elsewhere in the block of LD (2). Further investigation regarding SNPs in LD with rs2305480 in relation to pathophysiological pathways is therefore needed.

We conclude that 17q12 variant, rs2305480, is associated with asthma-like symptoms from the age of 2 to 4 years in preschool children and that smoke exposure already during fetal life, and exposure to ETS in infancy enhanced the effect of rs2305480. These effects were consistent in two independent, prospective, prenatally recruited birth cohorts.

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Authors contributions

RV, LD, MK, SW, AH, HM, HS, BB, DP, GK, VJ, and JJ involved in design, analysis, and writing of the article.

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Conflict of Interest

DS has received a consulting fee from Nycomed and was supported by Topinstitute Pharma and AstraZeneca for research projects relevant to this manuscript for which she served as co-investigator. GK and DP have received funding from Netherlands Asthma Foundation and ZonMW to support research projects relevant to this manuscript for which they served as principal investigator.

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