



Persistent Effects of Maternal Smoking during Pregnancy on Lung Function and Asthma in Adolescents

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Abstract

Rationale: The extent to which maternal smoking in pregnancy (MSP) has persisting effects on respiratory health remains uncertain and the mechanisms involved are not fully understood. Alterations in immune function have been proposed as a mechanism contributing to respiratory disease.

Objectives: To determine whether MSP increases risk of respiratory disorders in adolescence and, if so, whether this occurs by decreased lung function, altered immune function, and/or enhanced atopy.

Methods: Data on spirometry, bronchial responsiveness, respiratory symptoms, total and allergen-specific IgE and IgG4, immune function, and inflammatory markers were obtained from 1,129 participants in the 14-year follow-up of the Western Australian Pregnancy (Raine) Cohort and related to MSP using regression analyses.

Measurements and Main Results: MSP was reported for 21.0% (237 of 1,129) of participants, with 92 (8.1%) reporting current smoking. MSP was associated with some altered immune measures at age 14. MSP was strongly related to reduced lung function in current nonsmokers (forced expiratory flow midexpiratory phase [FEF_{25–75%}], $P = 0.016$; FEV₁/FVC, $P = 0.009$) and increased risk for current asthma (odds ratio [OR], 1.84; 95% confidence interval [CI], 1.16–2.92; $P = 0.01$), current wheeze (OR, 1.77; 95% CI, 1.14–2.75; $P = 0.011$), and exercise-induced wheeze (OR, 2.29; 95% CI,

1.37–3.85; $P = 0.002$), but not for bronchial hyperresponsiveness or atopy. Adjustment for immune measures and/or lung function in multivariate models did not greatly alter these associations and the increased risks for asthma and wheeze were not modified by sex, atopy, or maternal history of asthma or atopy.

Conclusions: MSP increases risk of asthma and wheezing in adolescence; mechanisms go beyond reducing lung function and exclude altering immune function or enhancing atopy.

Keywords: atopy; immune function; bronchial hyperresponsiveness; Raine study

At a Glance Commentary

Scientific Knowledge on the Subject: The impact of maternal smoking during pregnancy on lung function and asthma risks in children is well known. However, how long these effects persist and how they are mediated is not clearly understood.

What This Study Adds to the Field: The data from the present study show that maternal smoking during pregnancy increases risk for asthma and wheeze that persist into adolescence independent of effects on lower lung function, immune function, or allergic sensitization in adolescents.

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There is a great deal of evidence that maternal smoking during pregnancy (MSP) has detrimental effects on the health of children, including increased body mass index (BMI) (1–4), decreased lung function, and increased risk for development of asthma and wheeze (5–7). The mechanisms underlying these effects are uncertain; however, nicotine has reported detrimental effects on airway development (7). Prenatal tobacco smoke exposure is associated with a variety of epigenetic changes affecting gene expression, some of which are in keeping with an asthmatic phenotype (8, 9) and may be long lasting (8). Furthermore, it has been suggested that MSP can impair immune development and maturation, processes that begin *in utero* and continue after birth; these effects may modify risk for development of respiratory disease (10). MSP has been associated with a reduction in allergic sensitization in some cohorts, although there have been conflicting findings from different studies (11).

A previous study from our laboratory examining antenatal cytokines from 407 participants in the Western Australian Pregnancy (Raine) longitudinal birth cohort found that MSP was associated with lower concentrations of IL-4 and IFN- γ in cord blood and increased risk of wheeze and allergic sensitization at age 6 (12). This phenotype of low Th1/Th2 cytokine production observed in an unselected community cohort was also seen in infants at high genetic risk for atopy, who show delayed maturation of T-cell competence (13). Noakes and coworkers (14) examined the relationship between MSP and cord blood mononuclear cell cytokine responses and found that IL-13 production in response to house dust mite (HDM) or ovalbumin was higher in infants exposed to MSP ($n = 17$) than in unexposed ($n = 40$) neonates. They subsequently demonstrated in a different cohort that infants born to mothers who smoke ($n = 60$) showed significantly lower Toll-like receptor-mediated IL-6, tumor necrosis factor, and IL-10 production but higher IFN- γ production compared with infants born to mothers who do not smoke ($n = 62$) (15).

We have previously reported reduced lung function at birth in infants from the Raine cohort born to mothers who smoked during pregnancy (16) and have reported that prenatal and post-natal exposure to tobacco smoke in these children was associated with low lung function and

increased the risk of asthma and bronchial hyperresponsiveness (BHR) at age 6 (17, 18). The present study was undertaken to determine whether these negative impacts of MSP on respiratory health persisted into adolescence and if so whether these effects acted through any or all of reduced lung function; altered immune function, characterized by modified or blunted cytokine responsiveness at age 14; or enhanced atopy.

Methods

Study Subjects

Adolescents participating in the 14-year follow-up of the Raine Cohort (19) were studied. The cohort initially consisted of 2,860 live births and has been followed at birth, 1, 2, 3, 6, 8, 10, and 14 year of age and was recruited to participate in a randomized control trial of fetal monitoring. The prenatal study did not include any questions related to asthma or allergies or any childhood diseases, and no factors relevant to these were used to select the women for the study (see the online supplement for cohort description and Tables E1 and E2 in the online supplement). All aspects of the current study were approved by our institutional review board and parents gave written consent, with assent obtained from the teenagers. The antenatal questionnaire completed on enrolment at 16- to 18-week gestation asked about personal smoking and was used to assign MSP status to the adolescents. The reported smoking status indicated good agreement with serum cotinine on a random sample of the cohort (16).

Clinical and Immunologic Phenotyping at Age 14

Current asthma (17, 20) was defined as wheeze in the last 12 months in children with a doctor diagnosis of asthma ever. Children with current asthma who were using asthma medication were defined as having “medicated current asthma.” Spirometry was performed as described previously (18) according to American Thoracic Society guidelines. BHR was considered positive if FEV₁ fell by at least 20% on a standard methacholine challenge (17, 18).

Total IgE and specific IgE and IgG4 to a panel of allergens were measured in serum collected at age 6 and age 14 by

ImmunoCAP (Phadia AB, Uppsala, Sweden); the panel of allergens comprised HDM (*Dermatophagoides pteronyssinus*), rye grass pollen (rye; *Lolium perenne*), cat, couch grass (*Cynodon dactylon*), mold mix (*Penicillium notatum*, *Cladosporium herbarum*, *Aspergillus fumigatus*, *Candida albicans*, *Alternaria alternata*, *Helminthosporium halodes*), peanut, and food mix (egg white, milk, fish, wheat, peanut and soybean). Subjects were considered atopic if they had specific IgE greater than or equal to 0.35 kU/L for any of these allergens and/or total IgE greater than 300 kU/L (18).

Peripheral blood mononuclear cells were cultured (18) stimulated with HDM, rye, phytohemagglutinin, and LPS with and without IFN- γ , or poly(I:C). Cytokine proteins were measured by time-resolved fluorometry (IL-5, IL-10, IL-12, IL-13, IFN- γ , and tumor necrosis factor- α) or mRNAs were measured by quantitative reverse-transcriptase polymerase chain reaction (IL-4 and IL-9). Cytokine production above unstimulated control (delta) was calculated and used in analyses.

The mast cell and eosinophil products prostaglandin F_{2 α} and eosinophil protein X were measured from urine and normalized against creatinine using ELISA (Cayman Chemical, Ann Arbor, MI; Phadia AB). Soluble CD14 was measured in plasma by ELISA (BioScientific, Gympsea, Australia).

Statistics

The groups of clinical and immunologic variables used in the analyses are shown in Figure 1. The effects of MSP on clinical and respiratory outcomes were determined using chi-square or Mann-Whitney *U* tests as appropriate. Associations between MSP and binary clinical outcomes were examined using logistic regression. Because of the large number of immunologic measures, we summarized each set (antibodies, inflammatory markers, adaptive and innate stimuli responses) using the treelet transform (21) into three components per set and examined the associations of each and all of these with MSP and with respiratory outcomes using regression analyses. Because this method of examining possible mediation effects can be subject to various biases from both measured and unmeasured confounders (22), we further examined whether MSP acted through poor lung function by restricting analyses to those with

Antibodies	Inflammatory markers	In vitro cytokine responses	
Total IgE IgE & IgG4: HDM Rye Cat Couch Mould Peanut Food mix	soluble CD14 Prostaglandin F2a Eosinophil protein X Eosinophils Neutrophils Basophils Macrophages Lymphocytes	Adaptive stimuli: HDM, Rye, PHA IL-4 IL-5 IL-9 IL-10 IL-13 IFN γ	Innate stimuli: LPS+/-IFNγ, poly(I:C) IL-5 IL-10 IL-13 IFN γ IL-12

Figure 1. Matrix of variables examined in this study of the Western Australian Pregnancy (Raine) Cohort at age 14 years. HDM = house dust mite; PHA = phytohemagglutinin.

appropriate lung function. We also assumed that if there was no association between MSP and an intermediate variable or set of variables we would not further examine pathways through that set. Continuous variables were log₁₀-transformed except for maternal BMI, FEV₁/FVC, and forced expiratory flow midexpiratory phase (FEF_{25–75%})/FVC. Handling of confounders and possible mediation pathways is described in the online supplement.

Results

Results from questionnaires, lung function tests, and immunologic assays were available for 1,129 subjects seen at 14 years of age and comprise the study sample. MSP was reported for 21.0% (237 of 1,129) of the adolescents. MSP was strongly correlated with post-natal environmental tobacco smoke (ETS) exposure (*see* Table E3) and all MSP-positive subjects also had post-natal exposure to ETS. Ninety-two (8.1%) of the teenagers reported current smoking. One hundred and ten subjects (9.7%) fulfilled the definition of medicated current asthma and 124 (11.0%) had current asthma; 141 (12.5%) had current wheeze and 197 (17.4%) were positive for BHR. The prevalence of respiratory and allergic conditions at age 14 is shown in Table 1. Asthma (medicated current asthma, $P = 0.029$ and current asthma, $P = 0.002$) and current wheeze ($P = 0.006$) were more common among MSP-positive teenagers but BHR was not ($P = 0.37$). MSP-positive subjects also reported more exercise-induced wheeze ($P < 0.001$) and more had lower lung function (FEV₁/FVC $< 80\%$; $P = 0.004$), whereas the prevalence of atopy

($P = 0.59$) was not increased. Current smokers were excluded from further analyses because adjustment for current smoking would likely introduce bias because of its being on most postulated pathways from MSP (with odds ratio [OR] for current smoking from MSP of 3.2; 95% confidence interval [CI], 2.1–5.0) to respiratory outcomes. With (and also without, data not shown) adjustment for maternal asthma and maternal prepregnancy BMI (Table 2), MSP was a risk factor for current asthma (1.84 [1.16–2.92]; $P = 0.01$), current wheeze (1.77 [1.14–2.75]; $P = 0.011$), exercise-induced wheeze (2.29 [1.37–3.85]; $P < 0.002$), and poor lung function (2.00 [1.24–3.34]; $P = 0.005$). Differing effects of MSP by sex, maternal asthma status, and atopy were examined using interaction terms and none were significant ($P > 0.15$ in each analysis). MSP was associated with reduced lung function at age 14, including indices reflecting airway size and function, including lower FEF_{25–75%} ($P = 0.016$), lower FEV₁/FVC ($P = 0.009$), and FEF_{25–75%}/FVC ($P = 0.006$) (Table 3).

Few immunologic measures differed between MSP-positive and MSP-negative subjects (*see* Tables E4–E7). Total IgE titers were lower with MSP (geometric mean 61.1 vs. 74.0 kU/L; $P = 0.05$), as were titers of HDM-IgG4 (geometric mean 4.5 vs. 6.3 kU/L; $P = 0.03$) and Couch grass-IgG4 (geometric mean 0.01 vs. 0.05 kU/L; $P < 0.01$). In contrast, the number of peripheral circulating neutrophils (geometric mean 3.0×10^9 vs. 2.8×10^9 ; $P = 0.05$) and basophils (geometric mean 0.03 vs. 0.02×10^9 ; $P = 0.01$) was higher with MSP.

To examine whether the association of MSP with asthma outcomes was through a lung function pathway, models in Table 2

were reestimated including FEV₁/FVC % as an added explanatory variable (Table 4). There was only slight attenuation of the effects, indicating that MSP was unlikely to be acting solely through poorer lung function. Similar models including the major components from the treelet analyses of antibodies and inflammatory markers showed similar or slighter changes (Table 4). When analyses were done separately in participants with poor or adequate lung function, MSP effects were slightly weaker in those with good lung function (Table 4, *Model 4*) and stronger in those with poor lung function (data not shown). The lack of association between MSP and cytokine responses precluded examination of those pathways.

Discussion

MSP is a well-recognized risk factor for respiratory disease in children, but the precise mechanisms are largely speculative. The results of the present study confirm that this association persists into adolescence. Although MSP was associated with lower lung function, diminished lung function did not account for the increased risks for asthma and wheeze associated with MSP.

We have previously reported that children in this cohort born to mothers who smoked during pregnancy had a less mature immune system at birth, as judged from detectable levels of cytokines in cord blood, and that this was associated with an increased risk of asthma and atopy at 6 years of age (12). We had anticipated that MSP may increase asthma risk in adolescents by increasing allergic sensitization and enhancing allergy-related immune responses, independent of any effects on lung function. However, our data do not support this hypothesis. We saw little evidence of a less mature or reduced immune response in the present study. Total IgE and allergen-specific IgE and IgG4 levels tended to be lower with MSP, but most did not differ significantly between groups (*see* Table E4). The increased risks for asthma and wheeze associated with MSP seemed to be independent of adolescent immune function (Table 4). From the broad panel of adaptive and innate immune responses that we have measured in the current study, the only cytokine response that differed significantly according to MSP status was

Table 1: Prevalence of Respiratory Conditions and Atopy among 14-Year-Olds Stratified by Maternal Smoking in Pregnancy

Condition		Whole Population (n = 1,129)			Nonsmokers (n = 1,037)		
		MSP Pos N (%)	MSP Neg N (%)	P Value	MSP Pos N (%)	MSP Neg N (%)	P Value
Medicated current asthma* (n = 110)	Yes	32 (13.9)	78 (9.0)	0.029	27 (14.1)	72 (8.9)	0.031
	No	199	790		164	734	
Current asthma [†] (n = 124)	Yes	39 (16.9)	85 (9.8)	0.002	34 (17.8)	78 (9.7)	0.001
	No	192	783		157	728	
Current wheeze (n = 141)	Yes	42 (18.2)	99 (11.4)	0.006	37 (19.4)	90 (11.2)	0.002
	No	189	769		154	716	
Exercise-induced wheeze (n = 89)	Yes	33 (14.3)	56 (6.5)	<0.001	28 (14.7)	53 (6.6)	<0.001
	No	198	811		163	752	
Poor lung function [‡] (n = 77)	Yes	26 (11.0)	51 (5.7)	0.004	21 (10.8)	46 (5.6)	0.008
	No	211	841		173	782	
BHR (n = 197)	Yes	46 (19.4)	151 (16.9)	0.37	41 (21.1)	140 (16.9)	0.17
	No	191	741		153	688	
Atopy (n = 677)	Yes	138 (58.5)	539 (60.4)	0.59	115 (59.6)	506 (61.1)	0.67
	No	98	353		78	322	

Definition of abbreviations: BHR = bronchial hyperresponsiveness; MSP = maternal smoking during pregnancy.

Table shows numbers of subjects with and without conditions; prevalence was compared between MSP-positive (MSP Pos) and MSP-negative (MSP Neg) groups for each condition by chi-square test.

*Medicated current asthma: wheeze and asthma medication in last 12 months and doctor diagnosis of asthma ever.

[†]Current asthma: wheeze in last 12 months and doctor diagnosis of asthma ever.

[‡]Poor lung function was defined as FEV₁/FVC less than 80%.

IL-12 induced by LPS+IFN- γ , which was significantly lower with MSP. We have previously reported in this cohort that, among those individuals sensitized to HDM, IL-12 production reduces the risk of asthma and reduces asthma severity (18). Human *in vitro* studies (23) have shown that nicotine exposure affected dendritic cell maturation leading to reduced IL-12 production and activation of Th1 immune responses, an effect that was overcome only when environmental stimuli strongly supported a Th1 rather than Th2 response. Within our current study, IL-12 response did not modify risk for any of the outcomes examined. Thus, although it is possible that MSP and subsequent post-natal smoke exposure may directly reduce the capacity for IL-12 production, particularly in subjects with atopy characterized by high production of Th2 cytokines, our study does not suggest that MSP increases the risk for respiratory disorders via altered immune function in an unselected cohort of adolescents. We have previously reported that the timing of allergic sensitization is critical in increasing asthma risk associated with viral lower respiratory infections in early life (24, 25), in that the increased risk from lower respiratory infections in early life were only seen in those with contemporaneous allergic sensitization. No increased risk was seen in subjects who developed allergic sensitization later in life.

Thus, although there is no influence of MSP on allergic sensitization or on allergy-related immune responses in adolescents, this does not preclude this pathway being important in the first few years of life.

Although several previous studies have observed that deficient immune function in neonates or infants is linked to MSP or parental smoking in early life (12, 14, 15), to our knowledge only one has addressed whether this impaired immune function

persists into late childhood (26). Tebow and coworkers (26) examined the relationship between parental smoking after pregnancy and peripheral blood mononuclear cells production of IFN- γ and IL-4 in response to mitogens concavalin A and phorbol myristate acetate in 512 participants in the Tucson Children's Respiratory Study, who had been followed from the neonatal period until age 10 years. Dose-response relationships were observed between

Table 2: Associations between Maternal Smoking during Pregnancy and Respiratory Conditions at Age 14, Nonsmokers Only, Adjusted for Maternal Prepregnancy Body Mass Index and Maternal Asthma

Outcome	N	n	OR (95% CI)	P Value
Medicated current asthma*	898	99	1.49 (0.91–2.45)	0.11
Current asthma [†]	869	111	1.84 (1.16–2.92)	0.010
Current wheeze	854	126	1.77 (1.14–2.75)	0.011
Exercise-induced wheeze	899	80	2.29 (1.37–3.85)	0.002
Poor lung function [‡]	918	64	2.00 (1.14–3.50)	0.015
BHR [§]	807	175	1.31 (0.88–1.96)	0.18
Atopy	384	597	0.99 (0.71–1.37)	0.93

Definition of abbreviations: BHR = bronchial hyperresponsiveness; CI = confidence interval; N = total subjects included in logistic regression; n = subjects in regression with clinical outcome; OR = odds ratio.

*Medicated current asthma: asthma medication and wheeze in the past 12 months plus a doctor diagnosis of asthma ever.

[†]Current asthma: wheeze in the past 12 months plus a doctor diagnosis of asthma ever.

[‡]Poor lung function was defined as FEV₁/FVC less than 80%.

[§]BHR defined as FEV₁ fall of at least 20% with methacholine challenge.

^{||}Atopy defined as total IgE greater than 300 kU/L or specific IgE greater than or equal to 0.35 kU/L for any of seven allergens.

Table 3: Lung Function, Reported as % Predicted, Stratified by Maternal Smoking during Pregnancy Status in the Total Population and in Nonsmokers Alone

Measure	MSP	Total Population (n = 1,129)				Nonsmokers (n = 1,022)			
		N	Mean (SEM)	Mean Rank	P Value	N	Mean (SEM)	Mean Rank	P Value
% Predicted FEV ₁	Negative	889	96.3 (0.41)	561	0.598	825	96.4 (0.43)	511	0.774
	Positive	237	96.9 (0.84)	573		194	96.1 (0.93)	505	
% Predicted FVC	Negative	889	92.0 (0.41)	552	0.018	825	92.0 (0.42)	503	0.114
	Positive	237	94.3 (0.84)	608		194	93.7 (0.93)	540	
%Predicted FEF _{25-75%}	Negative	889	99.6 (0.70)	572	0.056	825	99.6 (0.72)	519	0.016
	Positive	237	96.7 (1.33)	526		194	95.6 (1.48)	463	
FEV ₁ /FVC, %	Negative	892	91.6 (0.22)	578	0.007	828	91.6 (0.22)	523	0.009
	Positive	237	90.1 (0.49)	514		194	89.9 (0.54)	461	
FEF _{25-75%} /FVC, %	Negative	892	1.10 (0.01)	579	0.006	828	1.10 (0.01)	524	0.006
	Positive	237	1.05 (0.02)	513		194	1.05 (0.02)	459	

Definition of abbreviations: FEF_{25-75%} = forced expiratory flow midexpiratory phase; MSP = maternal smoking during pregnancy; N = subjects in analysis.

parental smoking post-pregnancy (cumulative pack-years) and the risk of impaired IFN- γ responses in the child regardless of whether the mother smoked in pregnancy. In the present study we did not find reductions in allergen or mitogen-induced cytokine responses with MSP, with the exception of the IL-12 response referred to earlier, which, given the number of comparisons, may have been caused by chance.

The literature is divided on whether MSP or post-natal ETS exposure modifies risk for atopy in children, with different studies finding that smoke exposure increased risk (27–29), decreased risk (30, 31), or did not modify risk (32, 33). In some studies parental smoking only modified risk for atopy after stratification by maternal atopy (27, 28). In the present study we did not find an association between MSP and

atopy at age 14 in the entire cohort or after stratification for either maternal history or atopy or asthma. Our results suggest that MSP does not increase risk for current respiratory disorders in adolescence through increasing allergic sensitization.

Numerous studies have found that MSP and/or post-natal tobacco smoke exposure affects lung development, resulting in diminished lung function in childhood (34, 35). A previous report on the Raine cohort (16) demonstrated lower lung function at birth, as measured by peak tidal expiratory flow as a proportion of expiratory time, associated with MSP, with a significant dose–response found between peak tidal expiratory flow as a proportion of expiratory time and the daily number of cigarettes smoked. The data from the present study demonstrate lower lung function in adolescents born

to mothers who smoked during pregnancy. When examining lung function as a continuous variable, we found MSP effects on indices of airway caliber and function. FEF_{25-75%}, expressed as % predicted, was lower in the MSP group, especially when current smokers were excluded from the analysis (Table 3). In addition, we found both lower volume-corrected airway caliber, as judged from FEF_{25-75%}/FVC (36–39) and more airway obstruction, as judged from a lower FEV₁/FVC ratio, with MSP. Fetal exposure to nicotine via active placental transport has been shown in animal models to negatively affect lung and airway development, reducing surface complexity of the lung parenchyma, increasing collagen deposition in large and small airways, up-regulating surfactant protein gene expression, and inducing neuroendocrine cell hyperplasia

Table 4: Associations between Maternal Smoking during Pregnancy and Respiratory Conditions at Age 14, Nonsmokers Only, Adjusted for Maternal Prepregnancy Body Mass Index, Maternal Asthma, and Possible Intermediate Variables

Outcome	Model 1		Model 2		Model 3		Model 4	
	OR (95% CI)	P Value	OR (95% CI)	P Value	OR (95% CI)	P Value	OR (95% CI)	P Value
Medicated current asthma*	1.49 (0.91–2.45)	0.11	1.43 (0.87–2.36)	0.16	1.49 (0.88–2.55)	0.14	1.34 (0.77–2.31)	0.303
Current asthma [†]	1.84 (1.16–2.92)	0.010	1.76 (1.10–2.80)	0.018	1.77 (1.08–2.90)	0.024	1.74 (1.05–2.86)	0.030
Current wheeze	1.77 (1.14–2.75)	0.011	1.70 (1.09–2.65)	0.020	1.74 (1.08–2.79)	0.022	1.68 (1.05–2.71)	0.031
Exercise-induced wheeze	2.29 (1.37–3.85)	0.002	2.20 (1.31–3.70)	0.003	2.37 (1.37–4.09)	0.002	2.05 (1.17–3.59)	0.012

Definition of abbreviations: CI = confidence interval; OR = odds ratio.

Model 1: same as Table 2; Model 2: Model 1 plus FEV₁/FVC%; Model 3: Model 2 plus antibody components plus inflammatory marker components; Model 4: Model 1 restricted to participants with good lung function, defined as FEV₁/FVC greater than or equal to 80%.

*Medicated current asthma: asthma medication and wheeze in the past 12 months plus a doctor diagnosis of asthma ever.

[†]Current asthma: wheeze in the past 12 months plus a doctor diagnosis of asthma ever.

(7, 40). We were not able to examine separately the effects of prenatal and post-natal tobacco smoke exposure because of the high correlation between MSP and post-natal ETS exposure; all MSP-positive subjects also had post-natal ETS exposure (see Table E3). Most studies, including the present one, have difficulty in separating potential effects of prenatal from post-natal tobacco smoke exposure. The literature is unclear on whether prenatal or post-natal smoke exposure confers the most risk for asthma and associated respiratory disorders (41–45). A metaanalysis examining the effects of childhood ETS exposure on asthma development found that duration of exposure seems to be an important risk factor that has been underestimated by previous studies and metaanalyses (46). Unfortunately, we are not able to contribute definitive data to this debate from the present study.

One mechanism proposed in the literature for how tobacco smoke exposure increases the risk of respiratory diseases, including asthma, is via epigenetic modification of gene expression (47, 48). Epigenetics are presumed to underlie the observation of increased asthma in children whose maternal grandmother smoked during the mother's gestation (49). Although the effect was stronger if the mother smoked during the child's gestation a significant effect was also seen in children born to mothers who do not smoke (49).

An increase in global DNA methylation and increased methylation of promoter CpG islands has been reported in children born to mothers who smoked during pregnancy (8). We did not study gene methylation in the present study and cannot comment on any potential epigenetic effects on lung function because we did not study lung tissue. However, the lack of persistent effect of MSP on immune responses suggests that even if epigenetic effects were responsible for the effects on immune maturation we reported previously (12), they are not likely to be involved in conveying asthma risk in the teenagers studied.

MSP was associated with an increased risk for asthma and wheeze outcomes and lower lung function in the present study. Although MSP was strongly associated with low lung function, the adjusted analyses (Table 4) show that the effects of MSP on lung function do not account for the MSP-associated increase in asthma risk in adolescents. Both MSP and a reduction in FEV₁/FVC ratio were independent risk modifiers for current asthma, current wheeze, and exercise-induced wheeze. Because there are no major effects of MSP on atopy or asthma-related immune responses, immunologic mechanisms are unlikely to be responsible for the independent increase in asthma risk from MSP. We must point out that although MSP effects on immune mechanisms are

not responsible for the MSP effect on increasing asthma risk, this does not mean that immune factors impose no risk for asthma in adolescents. On the contrary, the usual "immune suspects" including allergen-specific IgE, markers of eosinophilic inflammation, and allergy-associated cytokine profiles are risk factors for asthma during adolescence in the Raine cohort (18).

In summary, MSP and subsequent exposure to ETS is associated with lower lung function and an independent increased risk of asthma that persists into adolescence. There was no effect of MSP on allergic sensitization or asthma-associated immune responses. Primary prevention of asthma and related respiratory conditions must include efforts to prevent MSP and post-natal ETS exposure. ■

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