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Original Research

ASTHMA

Do Grandmaternal Smoking Patterns Influence the Etiology of Childhood Asthma?

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Background: **Animal data suggest that tobacco smoke exposure of a mother when she is in utero influences DNA methylation patterns in her offspring and that there is an effect on the respiratory system, particularly airway responsiveness. The only study, to our knowledge, in humans suggests that there is a similar effect on asthma. The present study tests whether an association with respiratory problems can be confirmed in a large population study and aims to determine whether in utero exposure of the father has similar effects on his offspring.**

Methods: **Information from the Avon Longitudinal Study of Parents and Children was used to compare the offspring of women and of men who had themselves been exposed to cigarette smoke in utero; separate analyses were performed for children of women smokers and nonsmokers. The outcome measures were trajectories of history of early wheezing, doctor-diagnosed asthma by age 7 years, and results of lung function and methacholine challenge tests at 8 years. A variety of social and environmental factors were taken into account; offspring sexes were examined separately.**

Results: **There was no association with any outcome in relation to maternal prenatal exposure. There was some evidence of an increase in asthma risk with paternal prenatal exposure when the study mother was a nonsmoker (adjusted OR, 1.17; 95% CI, 0.97-1.41). This was particularly strong for girls (adjusted OR, 1.39; 95% CI, 1.04-1.86).**

Conclusions: **We did not find that maternal prenatal exposure to her mother's smoking had any effect on her children's respiratory outcomes. There was suggestive evidence of paternal prenatal exposure being associated with asthma and persistent wheezing in the granddaughters.**

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Abbreviations: ALSPAC = Avon Longitudinal Study of Parents and Children; AOR = adjusted OR; M = mother; MGM = maternal grandmother; − = did not smoke during pregnancy; PGM = paternal grandmother; + = smoked during pregnancy

The prevalence of asthma in childhood has increased over a relatively short period.[1] Consequently, although twin studies have indicated a strong genetic effect, environmental influences are assumed to have a strong influence. There is considerable evidence that gene-environment interactions may explain associations with both genetic and environmental influences.[2] Possible environmental exposures include tobacco smoke and other air pollutants.[2,3] Martino and Prescott[4] stated that "epigenetic paradigms are the likely mechanism behind the environment-driven epidemic of asthma" and pointed to cigarette smoke as being an important component of such an environment.

One possible mechanism, combining environmental and genetic effects, is an epigenetic influence on the development of asthma.[5] Evidence to support this is accumulating in animals and humans regarding cigarette smoking. For example, animal experiments have shown that (1) offspring of mice exposed to cigarette smoke during pregnancy had lower expression of

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Wnt genes[6] and of other genes involved in lung development[7]; (2) rats developed emphysematous lesions in their lungs in association with their grandmothers' exposure to nicotine when pregnant, regardless of whether this was via maternal or paternal prenatal exposure[8]; and (3) prenatal exposure of rats to nicotine resulted in reduced expression of peroxisome

proliferator-activated receptor-γ in the respiratory system of the offspring and in changes in respiratory responses to methacholine challenge; there were sex-specific effects, with male offspring exhibiting increased effects. The next generation also had the same response to methacholine challenges even though they had not been exposed to nicotine in utero themselves.[9] In humans (1) Breton and colleagues[10] showed specific methylation patterns of children whose mothers had smoked during pregnancy; (2) Murphy and colleagues[11] showed that exposure to maternal smoking in utero was associated with greater methylation levels at the *IGF2* gene region, especially in boys; and (3) a genome-wide study of 1,062 newborn infants showed differences in methylation patterns among those prenatally exposed; these involved *CYP1A1*, *GFI1*, and *AHRR*, with results that have been replicated in another cohort.[12]

The transgenerational findings in rats[8,9] raise the question as to whether there are similar intergenerational effects on human respiratory responses. One much-quoted study published in 2005 indicated that childhood asthma was influenced not only by prenatal smoking by the mother but also by the exposure of the mother in utero to her own mother's smoking.[13] We have been unable to identify any other human studies examining the grandmaternal history of smoking in the mother's pregnancy regarding asthma or lung development in her offspring. We have, therefore, analyzed the information from the population-based Avon Longitudinal Study of Parents and Children (ALSPAC) in an attempt to replicate the associations of Li and colleagues.[13] Various studies have shown a male-specific effect of exposure to smoking/nicotine in utero on development[9,14] and on gene methylation[9,11] in the offspring; we, therefore, hypothesized that effects would be more apparent in boys than girls. Thus, our primary aims were to test whether the maternal or paternal grandmother's prenatal smoking has an effect on measures relating to asthma and whether any effect is sex-specific.

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Materials and Methods

The data used in these analyses were collected as part of the ALSPAC, which was designed to assess the ways in which the environment interacts with the genotype to influence health and development.[15] Pregnant women, resident in the study area in southwest England with an expected date of delivery between April 1, 1991 and December 31, 1992, were invited to take part. About 80% of the eligible population did so.[16] Ethical approval for the study was obtained from the ALSPAC Law and Ethics Committee and the Local Research Ethics Committees.

Information collected from the parents during their study pregnancy included details of the maternal and paternal grandmothers. In this study we investigated the two pathways of possible influence of parental prenatal exposure to cigarette smoke on the study child.

The women and their partners were sent six questionnaires during pregnancy (e-Appendix 1; full details can be found on the study website http://www.bristol.ac.uk/alspac/researchers/data-access/data-dictionary/). Questions elicited information on their current smoking habits and those of their parents (ie, the study grandparents). If the parents had reported that their mothers had smoked, they were asked whether their mothers had smoked when they were pregnant with them—and, if so, were given the responses yes/no/don't know from which to select. Thus, the parents who replied "don't know" had a mother who smoked, but the parent was unsure whether she had smoked during her pregnancy. We have analyzed these data in two ways: (1) assuming that all these women did smoke during pregnancy and (2) omitting the "don't knows" from the analyses and only analyzing those definitely reporting smoking status during the study pregnancy (this we have treated as a sensitivity analysis).

Since maternal smoking in pregnancy has a well-demonstrated effect on the child's respiratory system,[17] we have analyzed mothers who themselves smoked during the study pregnancy separately from those who did not (smoked during pregnancy [+], did not smoke during pregnancy [−]). Consequently, we compare four groups of grandchildren: those whose grandmothers (maternal grandmothers [MGMs] and paternal grandmothers [PGMs]) smoked during the pregnancy resulting in their parent but whose mothers (Ms) had not smoked (MGM+M− with MGM−M− and PGM+M− with PGM−M−) and similar comparisons where the study mother herself smoked (MGM+M+ with MGM−M+ and PGM+M+ with PGM−M+).

Several different outcomes of respiratory function were used in this study:

1. The mother's report of doctor-diagnosed asthma ever in her study child at age 7 to 8 years in association with a history of wheezing in the preceding 12 months.
2. Three mutually exclusive trajectories of wheezing symptoms between the ages of 6 and 42 months, classified as early-onset transient (onset before 18 months but clear at 42 months), late onset (ie, no wheezing prior to 18 months, but present at 42 months), and early-onset persistent, persistent being defined as onset before 18 months and present at 42 months.[18]
3. Lung function measured by spirometry (Vitalograph 2120; Vitalograph) at age 8 to 9 years according to American Thoracic Society criteria.[19] Flow-volume curves were reviewed by one respiratory physician (J. H.) to ensure adherence to standards, resulting in the rejection of 338 measurements (4.6%) and the correction of 883 (11.5%), where the automated program had selected an inappropriate curve. Each variable (FEV_1, FVC, and maximal forced expiratory flow, midexpiratory phase) was converted to sex-, age-, and height-adjusted SD units using plots of residuals from multiple linear regression of lung function with sex, age, and height in

the ALSPAC cohort using the method described by Chinn and Rona.[20]

4. Airway responsiveness to methacholine was measured using the method of Yan et al.[21] Briefly, this involved using precalibrated, hand-held glass nebulizers (DeVilbiss No. 40; DeVilbiss Healthcare LLC) to deliver cumulative doubling doses of methacholine solution from approximately 0.03 μmol to a maximum dose of 4 μmol in eight steps at 1-min intervals. FEV_1 was measured 1 min after the previous dose (just prior to the subsequent dose delivery), and the test was continued until FEV_1 declined by > 20% from baseline or the maximum dose was given. FEV_1 was plotted against cumulative dose of methacholine, and a regression was fitted by least sum of squares to derive the dose-response slope (percent decline of FEV_1 per μmol methacholine) expressed for each subject. The intercept of this slope with a 20% reduction from baseline FEV_1 is equivalent to the provoking dose causing a 20% fall in FEV_1 of FEV_1 (percentage decline from baseline) per μmol methacholine for subjects who responded to bronchial challenge. Dose-response slopes were categorized as zero response or one of three tertiles of the distribution of non-zero slopes (mild, moderate, or severe airway responsiveness), each of which was categorized as % fall in FEV_1 from baseline per μmol of methacholine (greater value = more responsive); mild was defined as ≤ 0.815, moderate from 0.816 to 2.384, and severe as ≥ 2.385 (the severe tertile being equivalent to a provoking dose causing a 20% fall in FEV_1 ≤ 8.4 μmol).
5. Other data used in the analyses include the study mother's parity (ascertained from the maternal report of previous pregnancies resulting in either a live or stillbirth, and coded as 0; 1+); gestation (completed weeks: 39+; 37-38; ≤ 36); mother's partner smoking in pregnancy (primarily reported by partner, but maternal report was used if missing: yes, no); maternal age at the birth of the child (continuous); housing tenure as a measure of socioeconomic background (owned or mortgaged, rented public housing, all other); maternal education (highest level of educational attainment, in five levels of increasing achievement); whether the child was breastfed family history of asthma, defined as history in either parent at the time of the pregnancy; and the exposure of the child to environmental tobacco smoke at two time points (age 0-2 years and 2-8 years) measured as whether the child had been present in a room with someone smoking. For comparisons of MGM+M+ with MGM−M+, and of PGM+M+ with PGM−M+, the amount the mother smoked in pregnancy (grouped as 1-9, 10-19, 20+ cigarettes per day) was taken into account.

The data were analyzed using logistic regression for binary data (eg, diagnosed asthma) and using multiple regression for continuous scales (eg, measures of lung function). The analyses were undertaken in four stages. First, the unadjusted associations are given. Model I then adjusts for family history of asthma; model II additionally adjusts for gestation, parity, maternal education, breastfeeding, and the amount the mother smoked during pregnancy; model III additionally adjusts for paternal smoking in pregnancy and exposure of the child to environmental tobacco smoke at two time points. The analyses were repeated for boys and girls separately.

Results

The results of the analyses for each of the pairs of grandmaternal smoking history are shown in Table 1 for the mothers who themselves smoked in pregnancy (ie, MGM+M+ vs MGM−M+ and PGM+M+ vs PGM−M+). There is no evidence to suggest that prenatal exposure of the study mother or of the study father to cigarette smoke affected the likelihood of the study child being diagnosed with asthma. Similar lack of association was found for boys and for girls when analyzed separately. For children whose mothers did not smoke during pregnancy (MGM+M− vs MGM−M− and PGM+M− vs PGM−M−) (Table 2), there was a similar lack of evidence of an association with maternal prenatal exposure, but there was weak evidence of an association with paternal prenatal exposure (OR, 1.20; 95% CI, 1.00-1.43) that attenuated after adjustment (OR, 1.17; 95% CI, 0.97-1.41). When boys and girls were considered separately, there was stronger evidence for an association between paternal prenatal exposure (PGM+) and asthma in girls (adjusted OR [AOR] = 1.39; 95% CI, 1.04-1.86) than boys (AOR = 1.04; 95% CI, 0.81-1.34) (*P* interaction = .111).

Three different wheezing trajectories to age 42 months were considered: early-onset persistent, early-onset transient, and late onset. The only suggestion of an association with parental prenatal exposure was for persistent wheezers whose mothers did not smoke during the index pregnancy (e-Tables 1-12). Paternal prenatal exposure (PGM+) was associated with persistent wheezing in girls, (unadjusted OR = 1.60; 95% CI, 1.01-2.53; AOR = 1.42; 95% CI, 0.86-2.36]) (e-Table 9). Maternal prenatal exposure (MGM+) was associated with persistent wheezing (unadjusted OR = 1.41; 95% CI, 1.09-1.82), but this attenuated on adjustment (AOR = 1.26; 95% CI, 0.95-1.67); there was little difference in effect sizes between boys and girls (e-Tables 10-12). There were no consistent relationships between parental prenatal exposure (MGM+ or PGM+) and any measure of lung function (e-Tables 1-12).

Discussion

Using a large population study with detail on both maternal and grandmaternal smoking behavior during pregnancy, we have not found that maternal prenatal exposure (MGM+) to tobacco smoke has an adverse effect on her offspring's risk of respiratory symptoms or lung function during early childhood. In contrast, we have shown that prenatal tobacco smoke exposure of fathers (PGM+) was associated with an increased risk of persistent wheeze in early childhood and asthma by age 7 years in daughters of nonsmoking mothers in this population. However, there was little evidence of deleterious transgenerational effects of parental prenatal exposure on any objective measures of lung function, including bronchial responsiveness.

Table 1—*Risk of Doctor-Diagnosed Asthma by Age 7 y Among Children Whose Mothers Smoked During Pregnancy, According to Whether the Grandmother Smoked During the Pregnancy Resulting in the Parent of the Study Child*

Statistical Models[a]	PGM+M+ vs PGM−M+	MGM+M+ vs MGM−M+
Boys and girls		
No.	88 of 507 (PGM+M+), 94 of 540 (PGM−M+)	112 of 655 (MGM+M+), 126 of 770 (MGM−M+)
Unadjusted	1.00 (0.72-1.37)	1.05 (0.80-1.39)
Model I	1.02 (0.73-1.41)	1.05 (0.79-1.39)
Model II	0.99 (0.69-1.40)	1.09 (0.79-1.50)
Model III	0.94 (0.65-1.34)	1.08 (0.78-1.51)
Boys only		
No.	51 of 252 (PGM+M+), 50 of 279 (PGM−M+)	65 of 348 (MGM+M+), 70 of 389 (MGM−M+)
Unadjusted	1.16 (0.75-1.79)	1.05 (0.72-1.52)
Model I	1.19 (0.76-1.85)	1.02 (0.69-1.49)
Model II	1.18 (0.73-1.90)	1.12 (0.73-1.72)
Model III	1.07 (0.65-1.76)	1.10 (0.71-1.72)
Girls only		
No.	37 of 255 (PGM+M+), 44 of 261 (PGM−M+)	47 of 307 (MGM+M+), 56 of 381 (MGM−M+)
Unadjusted	0.84 (0.52-1.35)	1.05 (0.69-1.60)
Model I	0.84 (0.52-1.37)	1.09 (0.71-1.68)
Model II	0.86 (0.51-1.46)	1.05 (0.65-1.71)
Model III	0.84 (0.49-1.44)	1.07 (0.64-1.78)

Data shown are OR with 95% CI using the nonsmoking grandparents as the reference. M = mother; MGM = maternal grandmother; − = did not smoke during pregnancy; PGM = paternal grandmother; + = smoked during pregnancy.

[a]No. indicates the proportion of children who were given the diagnosis of asthma. Model I adjusts for family history of asthma; Model II additionally adjusts for gestation, parity, maternal education, breastfeeding, and the amount the mother smoked during pregnancy; Model III, in addition, adjusted for paternal smoking in pregnancy and exposure of the child to environmental tobacco smoke at two time points.

We failed to replicate the associations between the maternal prenatal smoking exposure and respiratory outcomes reported by Li and colleagues.[13] We have previously shown that maternal exposure in utero to her own mother's smoking (MGM+) resulted in a beneficial effect on her male offspring's birthweight but only if she was a nonsmoker herself (L. L. Miller, MSc; M. Pembrey, MD; G. Davey Smith, MD; K. Northstone, PhD; J. Golding, PhD; unpublished data, 2013). Conversely, here we find

Table 2—*Risk of Doctor-Diagnosed Asthma by Age 7 y Among Children Whose Mothers Did Not Smoke During Pregnancy, According to Whether the Grandmother Smoked During the Pregnancy Resulting in the Parent of the Study Child*

Statistical Models[a]	PGM+M− vs PGM−M−	MGM+M− vs MGM−M−
Boys and girls		
No.	244 of 1,723 (PGM+M−), 346 of 2,855 (PGM−M−)	231 of 1,689 (MGM+M−), 497 of 3,767 (MGM−M−)
Unadjusted	1.20 (1.00-1.43)[b]	1.04 (0.88-1.23)
Model I	1.23 (1.02-1.47)[b]	1.07 (0.90-1.26)
Model II	1.17 (0.97-1.41)	1.02 (0.85-1.22)
Model III	1.17 (0.97-1.41)	1.01 (0.84-1.22)
Boys only		
No.	133 of 854 (PGM+M−), 221 of 1,477 (PGM−M−)	138 of 853 (MGM+M−), 302 of 1,931 (MGM−M−)
Unadjusted	1.05 (0.83-1.32)	1.04 (0.84-1.30)
Model I	1.09 (0.86-1.38)	1.09 (0.87-1.36)
Model II	1.04 (0.81-1.34)	1.02 (0.81-1.30)
Model III	1.04 (0.81-1.34)	1.01 (0.79-1.28)
Girls only		
No.	111 of 869 (PGM+M−), 125 of 1,378 (PGM−M−)	93 of 836 (MGM+M−), 195 of 1,836 (MGM−M−)
Unadjusted	1.47 (1.12-1.92)[b]	1.05 (0.81-1.37)
Model I	1.48 (1.12-1.95)[b]	1.05 (0.80-1.37)
Model II	1.40 (1.05-1.87)[b]	1.01 (0.76-1.33)
Model III	1.39 (1.04-1.86)[b]	1.03 (0.77-1.37)

Data shown are OR with 95% CI using the nonsmoking grandparents as the reference. See Table 1 legend for expansion of abbreviations.

[a]No. indicates the proportion of children who were given the diagnosis of asthma. Model I adjusts for family history of asthma; Model II additionally adjusts for gestation, parity, maternal education and breastfeeding; Model III, in addition, adjusted for paternal smoking in pregnancy and exposure of the child to environmental tobacco smoke at two time points.

[b]Result is statistically significant.

that there are no convincing effects of maternal prenatal exposure on signs of the offsprings' respiratory symptoms or measurements. We considered the relationship for boys and girls separately and for mothers who smoked in the study pregnancy compared with those who did not, but found no consistent relationships. There were a number of differences between the two studies that may possibly explain this.

1. The numbers in the study by Li and colleagues[13] were smaller (279 cases, 412 control subjects) compared with the present study (966 cases of asthma, 5,915 without asthma).
2. The study by Li and colleagues[13] adjusted only for gestation, passive smoking, and race; the present study comprised 96% white children; analyses adjusted for gestation, passive smoking, family history of asthma, parity, maternal education, paternal smoking in pregnancy, and, for smoking mothers, the amount the mother smoked. The latter was particularly important, as mothers whose own mother smoked during pregnancy are more likely to be heavy smokers themselves.[22]
3. The background environment, and, hence, the influence on the development of asthma, is likely to have differed substantially between Southern California and England and may conceivably have been responsible for the differences between the findings in the two studies.
4. We think it unlikely, but not impossible, that a difference in the ages studied may have been relevant: the study by Li and colleagues[13] was concerned with asthma in the first 5 years, whereas our study was concerned with asthma diagnosed by 7 to 8 years of age.

It is of relevance to note that the wheezing trajectory analysis, which considers only the first 42 months, did show an association between maternal in utero exposure and persistent wheezing, although this association was attenuated on full adjustment for age, education level and parity of the mother, paternal smoking, housing tenure, whether the child was breastfed, parental history of asthma, and exposure of the child to environmental tobacco smoke, and also, if the mother smoked in pregnancy, the amount smoked.

There have been a number of studies that have indicated more extreme effects of prenatal smoke exposure on the developing boy compared with the girl; these include a greater association with intrauterine growth[14,23] and congenital defects.[24,25] Additionally, prenatal exposure to nicotine can interfere with the development of the male gonadal axis and with the organization of sexually dimorphic behavior.[26] It is conceivable that the adverse effects of paternal prenatal smoke exposure may be more important than maternal prenatal smoke exposure in determining the risk of transgenerational effects. Indeed, if the mechanism operates through epigenetic mechanisms, there is evidence that epigenetic consequences of prenatal exposure may be more evident in male than female offspring.[11]

There are a number of key strengths in this study: (1) the data on grandmaternal smoking in pregnancy were ascertained from the parents during the study pregnancy, prior to the birth of the study child, and, thus, are not biased by identification of respiratory outcomes; (2) data on respiratory outcomes were collected using different methods, including maternal reports asked at various time points, and objective measurements of lung function and bronchial responsiveness to methacholine; and (3) the number of subjects with available data were large, enabling a detailed analysis of different exposure subgroups defined a priori.

Nevertheless, there are potential difficulties with this study: (1) we relied on parental reporting of their mothers' smoking habit, which was subject to reporting bias. However, the study method of obtaining information using postal questionnaires allowed time for each parent to acquire the relevant answer from members of their family. (2) As with all observational studies, there is the possibility that all appropriate confounders have not been taken into account and residual confounding exists.

Conclusions

We found no association between asthma risk and maternal exposure in utero; however, sex-specific analysis did indicate that paternal exposure to his mother smoking during pregnancy was associated with a higher asthma risk in his daughters. These results should be regarded as hypothesis-generating only, being dependent just on reported symptoms without corroborating biologic evidence.

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Ms Miller: contributed to statistical analyses and writing and editing of the manuscript.
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Dr Pembrey: contributed to the original concept and writing and editing of the manuscript.
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Additional information: The e-Appendix and e-Tables can be found in the "Supplemental Materials" area of the online article.

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