The prevalence of asthma in childhood has increased over a relatively short period. 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. Possible environmental exposures include tobacco smoke and other air pollutants. Martino and Prescott 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. 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 Wnt genes and of other genes involved in lung development; (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; and (3) prenatal exposure of rats to nicotine resulted in reduced expression of peroxisome...
proliferator-activated receptor-\(\gamma\) 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 genomewide study of 1,062 newborn infants showed differences in methylation patterns among those prenatally exposed; these involved \(CYP1A1\), \(GFI1\), and \(AHR\), 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.\(^{14}\) Various studies have shown a male-specific effect of exposure to smoking/nicotine in utero on development\(^{15}\) and on gene methylation\(^{11}\) 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.

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.\(^{16}\) 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 know” 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 86 (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

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**Funding/Support:** The statistical analyses for this project were undertaken with funding from the Medical Research Council [Grant G1100226].

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DOI: 10.1378/chest.13-1371
4. Airway responsiveness to methacholine was measured using the method of Yan et al.21 Briefly, this involved using precali-
brated, 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. FEV₁ was measured 1 min after the previous dose (just prior to the subsequent dose delivery), and the test was con-
tinued until FEV₁ declined by >20% from baseline or the maximum dose was given. FEV₁ was plotted against cumu-
lative dose of methacholine, and a regression was fitted by least
sum of squares to derive the dose-response slope (percent decline of FEV₁ per μmol methacholine) expressed for
each subject. The intercept of this slope with a 20% reduc-
tion from baseline FEV₁ is equivalent to the provoking dose causing a 20% fall in FEV₁ of FEV₁ (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 catego-
rized as % fall in FEV₁ from baseline per μmol of metha-
choline (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 provok-
ing dose causing a 20% fall in FEV₁ ≤ 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+ vs MGM+M-, and
of PGM+M+ vs PGM+M-, the amount the mother
smoked in pregnancy (grouped as 1-9, 10-19, 20+ ciga-
rettes per day) was taken into account.

The data were analyzed using logistic regression for binary data
(e.g. diagnosed asthma) and using multiple regression for con-
tinuous scales (e.g. measures of lung function). The analyses
were undertaken in four stages. First, the unadjusted associa-
tions are given. Model I then adjusts for family history of asthma; model II
additional adjusts for gestation, parity, maternal education, breast-
feeding, 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 the pairs of the
parents 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 (MG+M– vs
MG–M– and MG+M– vs MG–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 pre-
natal exposure (OR, 1.20; 95% CI, 1.00-1.43) that attenu-
ated 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 in-
teraction = .111).

Three different wheezing trajectories to age
42 months were considered: early-onset persistent,
early-onset transient, and late onset. The only sugges-
tion 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, 1.01-2.35; AOR = 1.42; 95% CI, 0.86-2.36] (e-
Table 9). Maternal prenatal exposure (MG+M+) 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
(MG+M+ or PGM+) and any measure of lung func-
tion (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 (MG+M+) 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.
During Pregnancy, According to Whether the Grandmother Smoked During the Pregnancy Resulting in the Parent of the Study Child

<table>
<thead>
<tr>
<th>Statistical Models a</th>
<th>PGM + M+ vs PGM - M+</th>
<th>MGM + M+ vs MGM - M+</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Boys and girls</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No.</td>
<td>88 of 507 (PGM + M+), 94 of 540 (PGM - M+)</td>
<td>112 of 655 (MGM + M+), 126 of 770 (MGM - M+)</td>
</tr>
<tr>
<td>Unadjusted</td>
<td>1.00 (0.72-1.37)</td>
<td>1.05 (0.80-1.39)</td>
</tr>
<tr>
<td>Model I</td>
<td>1.02 (0.73-1.41)</td>
<td>1.05 (0.79-1.39)</td>
</tr>
<tr>
<td>Model II</td>
<td>0.99 (0.69-1.40)</td>
<td>1.00 (0.79-1.50)</td>
</tr>
<tr>
<td>Model III</td>
<td>0.94 (0.65-1.34)</td>
<td>1.06 (0.78-1.51)</td>
</tr>
<tr>
<td><strong>Boys only</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No.</td>
<td>51 of 252 (PGM + M+), 50 of 279 (PGM - M+)</td>
<td>65 of 348 (MGM + M+), 70 of 389 (MGM - M+)</td>
</tr>
<tr>
<td>Unadjusted</td>
<td>1.16 (0.75-1.79)</td>
<td>1.05 (0.72-1.52)</td>
</tr>
<tr>
<td>Model I</td>
<td>1.19 (0.76-1.85)</td>
<td>1.02 (0.69-1.49)</td>
</tr>
<tr>
<td>Model II</td>
<td>1.18 (0.73-1.90)</td>
<td>1.12 (0.73-1.72)</td>
</tr>
<tr>
<td>Model III</td>
<td>1.07 (0.65-1.76)</td>
<td>1.10 (0.71-1.72)</td>
</tr>
<tr>
<td><strong>Girls only</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No.</td>
<td>37 of 255 (PGM + M+), 44 of 261 (PGM - M+)</td>
<td>47 of 307 (MGM + M+), 56 of 381 (MGM - M+)</td>
</tr>
<tr>
<td>Unadjusted</td>
<td>0.84 (0.52-1.35)</td>
<td>1.05 (0.69-1.60)</td>
</tr>
<tr>
<td>Model I</td>
<td>0.84 (0.52-1.37)</td>
<td>1.00 (0.71-1.68)</td>
</tr>
<tr>
<td>Model II</td>
<td>0.86 (0.51-1.46)</td>
<td>1.05 (0.65-1.71)</td>
</tr>
<tr>
<td>Model III</td>
<td>0.84 (0.49-1.44)</td>
<td>1.07 (0.64-1.78)</td>
</tr>
</tbody>
</table>

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

aNo. 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; 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. 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. Fembrey, 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

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

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

aNo. 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.
bResult 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.

Acknowledgments

Author contributions: Dr Golding is the guarantor of the manuscript and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Ms Miller: contributed to statistical analyses and writing and editing of the manuscript.

Dr Henderson: contributed to writing and editing of the manuscript.

Dr Northstone: contributed to statistical analyses and writing and editing of the manuscript.

Dr Pembrey: contributed to the original concept and writing and editing of the manuscript.

Dr Golding: contributed to the original concept and writing and editing of the manuscript.

Financial/nonfinancial disclosures: The authors have reported to CHEST that no potential conflicts of interest exist with any
companies/organizations whose products or services may be discussed in this article.

**Role of sponsors:** The sponsor had no role in the design of the study, the collection and analysis of the data, or the preparation of the manuscript.

**Other contributions:** We thank all the families who took part in this study, the midwives for their help in recruiting them, and the Avon Longitudinal Study of Parents and Children team, which includes interviewers, computer and laboratory technicians, clerical workers, research scientists, volunteers, managers, receptionists, and nurses. The UK Medical Research Council (MRC), the Wellcome Trust, and the University of Bristol currently provide core support for the study.

**Additional information:** The e-Appendix and e-Tables can be found in the "Supplemental Materials" area of the online article.

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**REFERENCES**


