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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

For editorial comment see page 1182

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