

The sins of the mothers: does grandmaternal smoking influence the risk of asthma in children?

A John Henderson

Visiting the iniquity of the fathers upon the children, and upon the children's children, unto the third and to the fourth [generation].

Exodus 34:7, King James Bible

There can be few readers of *Thorax* who remain unaware of the body of evidence that supports a detrimental effect of tobacco smoking by pregnant women on the subsequent health and development of their offspring. Childhood disorders associated with mothers smoking when pregnant include low birth weight, sudden unexplained death in infancy, asthma¹ and a number of neurobehavioural outcomes, although whether all these reported associations are truly causal has been questioned. Such outcomes could be ascribed to direct toxic effects of tobacco smoke constituents on the fetoplacental unit with consequences for fetal organ development which might manifest through the life course. For example, intrauterine exposure to maternal smoking is associated with low lung function² and increased respiratory symptoms³ in infancy and, through reduced birth weight, low FEV₁ and COPD in adulthood.⁴ However, in seeking the developmental origins of respiratory disease, we now have to extend our horizons to more distant ancestry, when lifestyle and behavioural practices now recognised to be harmful were socially (and medically) acceptable. In this issue of *Thorax*, Maria Magnus and colleagues report an association between grandmothers' smoking when pregnant and an increased risk of asthma in their grandchildren even when the mother herself did not smoke when pregnant with the index child.⁵

These results came from the analysis of observational data in the Norwegian Mother and Child Cohort Study (MoBa), a very large, prospective birth cohort of around 100 000 women and their children with asthma outcomes ascertained from questionnaires and from linkage to a

national prescribing registry. Between 25 000 and 45 000 children had reached the age of 7 years and had asthma outcome status available for analysis; either through questionnaire responses or being registered as taking prescribed medicine for asthma. Grandmaternal smoking was reported by the mother, with clear potential for recall bias, and in the absence of direct data about grandparental lifestyle and health status, the possible confounding of associations was modelled on maternal characteristics as a proxy for these. Data were presented for the maternal lineage only with no information on paternal or grandpaternal smoking reported. What the study found was an increased relative risk of reported asthma and use of asthma medications at age 7 years when the grandmother smoked during pregnancy with the study mother. The effect estimates were consistent for both the reported and registry-based outcomes but attenuated somewhat when they were adjusted for maternal variables, including her own pregnancy smoking history. Interestingly, when the results were stratified according to whether or not the mother had smoked when pregnant, there was little difference in adjusted risks and the grandmaternal effect was at least as strong as when only the mother had smoked during pregnancy with no grandmaternal exposure. Another notable observation was that there was no difference in birth weights according to whether or not the grandmother had smoked when pregnant with the child's mother. The authors with appropriate circumspection have suggested that these results could be explained by transgenerational effects mediated through epigenetic changes associated with tobacco smoke exposure.

Of course there are several other possible explanations for these findings and the authors have shown due diligence by expounding each of them in some detail. One of these is clustering of confounders within families, as exemplified by their reliance on maternal proxies for grandmaternal characteristics. However, if this is a true observation, the question is whether this intergenerational effect can be

explained by the epigenetic modification of DNA that is heritable. Others have sought evidence of transgenerational influences of smoking on asthma risk. In a case-control study nested in the Californian Children's Health Study, the authors confirmed an increased risk of asthma in children whose mothers smoked during pregnancy and reported an increased risk when the mother reported that she had been exposed to her own mother's smoking during pregnancy.⁶ In contrast to these two studies, the British Avon Longitudinal Study of Parents and Children (ALSPAC) had information on both maternal and paternal prenatal exposure to grandmaternal smoking. In this cohort, Miller and colleagues reported no influence of maternal prenatal exposure on her child's asthma risk, whether or not she smoked when pregnant.⁷ However, a sex-specific effect on increased girls' asthma risk was associated with paternal exposure to grandmaternal smoking but only when the mother did not smoke when pregnant.

There is a gathering tsunami of interest in the concept that the epigenetic modification of DNA could provide an explanation for inheritance of traits through mechanisms other than coding sequence. Several mechanisms exist that can regulate DNA expression and which are susceptible to modification through exposure to environmental influences, such as tobacco smoke. These include DNA methylation, histone modification and RNA-associated silencing, of which DNA methylation has been studied most extensively in humans. This refers to the binding of methyl (CH₃) groups to cytosine residues that are usually adjacent to a guanine nucleotide (CpG sites) forming 5-methylcytosine and leading to gene silencing. Although a high proportion of CpG sites throughout the genome are methylated, there are clusters of CpG-rich sites (CpG islands) in many promoter, locus control and initial exon regions that are generally less methylated and available for transcription. Changes in DNA methylation at several sites across the genome have been reported in association with tobacco smoking⁸ and, in the MoBa study, the differential methylation of CpG sites in DNA from cord blood of infants whose mothers smoked during pregnancy has been described.⁹ In the Children's Health Study, both global and site-specific methylation patterns in buccal DNA obtained during childhood were reported to differ between children exposed and unexposed to prenatal maternal smoking.¹⁰ Furthermore, there was evidence of modification of these effects

Correspondence to Professor John Henderson, School of Social and Community Medicine, ALSPAC, University of Bristol, Oakfield House, Bristol BS8 2BN, UK; a.j.henderson@bristol.ac.uk

by glutathione-S-transferase gene polymorphisms (*GSTM1* and *GSTP1*), which have been previously reported to interact with maternal smoking in association with asthma-related outcomes.^{11 12} Differential methylation of cord blood DNA has also been related to birth weight of human infants^{13 14} and the methylation of specific genes was reported to be additive to the effects of maternal smoking on birth weight percentile.¹⁵ However, although these data are supportive of tobacco smoke exposure modifying the epigenomic signature of DNA, they do not yet provide the proof of transgenerational inheritance of these effects. In considering the case of grandmaternal smoking it is important to differentiate the impact of in utero exposure on the developing embryo and its germline (which will eventually produce grandchildren) and transgenerational effects that may be observed in generations that were not exposed to the initial stimulus. None of the observational studies of the apparent influence of grandparental smoking on asthma in their grandchildren cited here fulfil this latter criterion.

One of the barriers to the propagation of transgenerational effects of environmental influences on the epigenome is the germline reprogramming that occurs in mammals, removing epigenetic signatures that have been acquired through programming, accident or exposure to environmental toxins.¹⁶ This occurs in both the germline and in the zygote immediately after fertilisation rendering very low the probability that epigenetic marks will be inherited except in imprinted loci that are resistant to postzygotic reprogramming. Despite outstanding question about heritability, however, the study of epigenetic mechanisms offers much promise in helping to explain the bridge between genotype and phenotype in asthma. It has long been recognised that environmental influences are important determinants of asthma and epigenetic marks offer an insight into how environment can influence the expression of genes in key biological pathways underpinning asthma (reviewed by Kabisch¹⁷). A Spanish study based on two independent cohorts in the Infancia y Medio Ambiente project reported the hypomethylation of the *ALOX12* gene in association with persistent but not transient wheezing in early childhood compared with never

wheezers.¹⁸ This was present in DNA collected at birth and age 4 years and was associated with levels of maternal exposure to the persistent organic pollutant dichlorodiphenyldichloroethylene measured in serum in early pregnancy but not with pregnancy smoking history or folate intake; a methyl donor.¹⁹ Therefore, patterns of epigenetic marks such as DNA methylation could act as biomarkers of specific disease phenotypes enabling progress towards personalised approaches to asthma classification and treatment and, as they are themselves modifiable, may eventually give rise to tractable targets for disease modification or prevention.²⁰ Observational studies hinting at the transgenerational inheritance of epigenetic marks such as the one reported by Magnus and colleagues give a glimpse of the potential for epigenetics to explain observable biological phenomena. With many of the world's leading phenotypic resources acquiring genomewide methylation data the challenge ahead is to exploit this rapidly accumulating wealth to bring about a step change in our thinking about the origins, treatment and prevention of complex, polygenic respiratory diseases.

Competing interests None.

Provenance and peer review Commissioned; internally peer reviewed.



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To cite Henderson AJ. *Thorax* 2015;**70**:207–208.

Received 31 December 2014

Revised 8 January 2015

Accepted 14 January 2015

Published Online First 4 February 2015



► <http://dx.doi.org/10.1136/thoraxjnl-2014-206438>

Thorax 2015;**70**:207–208.

doi:10.1136/thoraxjnl-2014-206438

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