28. Thomsen SF. Genetics of asthma: an introduction for the clinician. *Eur Clin Respir J* 2015;**2**:24643.
29. Lim RH, Kobzik L, Dahl M. Risk for asthma in offspring of asthmatic mothers versus fathers: a meta-analysis. *PLoS One* 2010;**5**:e10134.
30. Paaso EM, Jaakkola MS, Lajunen TK, Hugg TT, Jaakkola JJ. The importance of family history in asthma during the first 27 years of life. *Am J Respir Crit Care Med* 2013;**188**:624–26.
31. Lim RH, Kobzik L. Maternal transmission of asthma risk. *Am J Reprod Immunol* 2009;**61**:1–10.
32. Guilbert TW, Stern DA, Morgan WJ, Martinez FD, Wright AL. Effect of breastfeeding on lung function in childhood and modulation by maternal asthma and atopy. *Am J Respir Crit Care Med* 2007;**176**:843–48.
33. Raby BA, Van Steen K, Celedón JC, Litonjua AA, Lange C, Weiss ST. Paternal history of asthma and airway responsiveness in children with asthma. *Am J Respir Crit Care Med* 2005;**172**: 552–58.
34. Rogers JM. Tobacco and pregnancy. *Reprod Toxicol* 2009;**28**: 152–60.
35. Bisht S, Faiq M, Tolahunase M, Dada R. Oxidative stress and male infertility. *Nat Rev Urol* 2017;**14**:470–85.
36. Maritz GS, Dennis H. Maternal nicotine exposure during gestation and lactation interferes with alveolar development in the neonatal lung. *Reprod Fertil Dev* 1998;**10**:255–61.
37. Sekhon HS, Jia Y, Raab R *et al*. Prenatal nicotine increases pulmonary alpha7 nicotinic receptor expression and alters fetal lung development in monkeys. *J Clin Invest* 1999;**103**:637–47.
38. Sekhon HS, Keller JA, Benowitz NL, Spindel ER. Prenatal nicotine exposure alters pulmonary function in newborn rhesus monkeys. *Am J Respir Crit Care Med* 2001;**164**:989–94.
39. Skinner MK. Endocrine disruptor induction of epigenetic transgenerational inheritance of disease. *Mol Cell Endocrinol* 2014;**398**:4–12.
40. Arshad SH, Karmaus W, Zhang H, Holloway JW. Multigenerational cohorts in patients with asthma and allergy. *J Allergy Clin Immunol* 2017;**139**:415–21.
41. Perera F, Herbstman J. Prenatal environmental exposures, epigenetics, and disease. *Reprod Toxicol* 2011;**31**:363–73.
42. Rehan VK, Liu J, Naeem E *et al*. Perinatal nicotine exposure induces asthma in second generation offspring. *BMC Med* 2012;**10**:129.
43. Patil VK, Holloway JW, Zhang H *et al*. Interaction of prenatal maternal smoking, interleukin 13 genetic variants and DNA methylation influencing airflow and airway reactivity. *Clin Epigenetics* 2013;**5**:22.
44. Pembrey ME, Bygren LO, Kaati G *et al*. Sex-specific, male-line transgenerational responses in humans. *Eur J Hum Genet* 2006;**14**:159–66.
45. Forster P, Hohoff C, Dunkelmann B, *et al*. Elevated germline mutation rate in teenage fathers. *Proc Biol Sci* 2015;**282**:20142898.
46. Linschooten JO, Van Schooten FJ, Baumgartner A *et al*. Use of spermatozoal mRNA profiles to study gene-environment interactions in human germ cells. *Mutat Res* 2009;**667**:70–76.
47. Marczylo EL, Amoako AA, Konje JC, Gant TW, Marczylo TH. Smoking induces differential miRNA expression in human spermatozoa: a potential transgenerational epigenetic concern? *Epigenetics* 2012;**7**:432–39.
48. Amanai M, Brahmajosyula M, Perry AC. A restricted role for sperm-borne microRNAs in mammalian fertilization. *Biol Reprod* 2006;**75**:877–84.
49. Greenland S. Quantifying biases in causal models: classical confounding vs collider-stratification bias. *Epidemiology* 2003;**14**: 300–6.

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Commentary: Tobacco smoking and asthma: multigenerational effects, epigenetics and multilevel causal mediation analysis

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In this issue of the *Journal*, Accordini *et al*. document the findings from their study of the multigenerational asthma effects of tobacco smoking.[1] They found that grandmothers' tobacco smoking during pregnancy was

associated with their own children's asthma, and that the mothers' smoking was associated with asthma in the grandchildren. Importantly, they found that the grandmothers' smoking (in generation 1) when pregnant with the mothers (in generation 2) was linked to the grandchildren's asthma with nasal allergies (in generation 3), through pathways other than through the mothers' asthma or smoking during pregnancy. This is not entirely surprising. There is growing evidence that phenotypic risk factors can be subject to vertical or multigenerational inheritance. Furthermore, tobacco smoking is detrimental to health, having been linked to substantial morbidity and mortality from cancer, respiratory disease and heart disease, among others.[2] In pregnancy, tobacco smoking is known to lead to poor perinatal, pediatric and life-long outcomes, such as birth outcomes including low birth weight, small-for-gestational age, birth defects and many others.

The vertical transmission of the asthma effects of smoking within the maternal line will, at first, seem to suggest another reason for stopping or not initiating smoking during pregnancy in (grand)mothers. It is unclear whether telling smokers that their tobacco smoking will have deleterious health effects in their progeny will prove effective. Similar arguments could be made for the 'transmission' of the asthma effect of fathers' smoking during their reproductive development. Nonetheless, it is important to learn about the multigenerational asthma effects of smoking for the reasons outlined by Accordini *et al.*[1] The study provides important evidence that grandparents' health behaviour can have a lasting impact on their children's and grandchildren's health and, perhaps, further down the line. If this suggested mechanism proves to be durable, it offers an important early window for tackling risk factors of asthma. This study supports an epigenetic mechanism whereby (nicotine from) tobacco smoking leads to epigenetic variations that are transmitted from grandmothers to their grandchildren, through pathways other than through asthma phenotypes in their children.[3–5] Epigenetics has been gaining focus in studies of human health and disease, including asthma.[3–9] The study findings indicate the need for further investigation of epigenetic and other mechanisms that could be responsible for the links between ancestral tobacco smoking and asthma in descendants.

The authors should be commended for their thoughtfully executed study, especially for their multicenter, multilevel, multigenerational, multiple-exposure, single-mediator design and analysis, coupled with sensitivity analysis for uncontrolled confounding (see also the Supplementary Appendix of Accordini *et al*).[1] The study explored possible explanations of the multigenerational signals it found: multigenerational genetic, epigenetic or environmental mechanisms. The explanations also considered implications of information bias, collider-stratification bias due to unmeasured confounder(s) of mediator–outcome relations, and uncontrolled confounding of exposure–outcome relations. The authors[1] were rightly worried that such biases could explain part or all of their results. They used causal graphical theory to guide their choice of variables for confounding control for their assumed data generating process.[10,11] They also conducted probabilistic bias analysis[12,13] to assess the sensitivity of their results to an unmeasured common cause of the exposures, mediator and outcome. Taken together, these are important developments for a multigenerational epidemiological study.

What should we expect from future studies on this topic? First, we need more large multigenerational studies with prospectively collected repeated measurements on exposures, mediators, epigenetic markers, covariates and outcomes from diverse populations around the world. Clever, multistage designs with committed funding will be needed for feasible and well powered studies.

Second, we need modern mediation analysis with an eye on path-specific and heterogeneous effects to shed light on mechanisms involved in the multigenerational links from smoking to asthma phenotypes.[14–17] Attention should also be paid to the complexities of identification and estimation of mediated effects in multilevel, multiple-exposure, multiple-mediator and multiple-outcome studies of multigenerational effects of tobacco smoking and other exposures. I propose the use of a multiple-exposure, multiple-mediator and multiple-outcome (MEMMMO) framework in studies of multigenerational epigenetic inheritance. A well-developed MEMMMO framework consists of a structural causal model of the connections and the assumptions needed to identify and estimate the multiple mediational and interaction effects of multiple exposure interventions on multiple outcomes that have complex links over time. In this framework, effect decomposition for mediation analysis should consider other types of direct and indirect effects beyond controlled direct effects in multigenerational studies whenever the assumptions for natural, controlled or interventional effect decomposition appear defensible.[14,15] The causal mediation analysis of these studies within a MEMMMO framework will require further methods and software development, given the current limitations in the literature.[14,17–22]

Third, future studies should undertake multiple-bias modeling to address the impact of different combinations of uncontrolled confounding, selection bias and information bias on study results and conclusions.[12,14,23–29] Multiple-bias modeling can involve probabilistic sensitivity analysis conducted using Monte Carlo simulations and can be subsumed under an integrated general approach to causal mediation analysis (namely, g-computation via

Monte Carlo simulation) and record-level bias analysis (namely, generalized bias simulation, again using Monte Carlo methods).[15,23]

Indeed, epigenetic investigations can and should benefit from modern causal inference methods and conduct more mediation, interaction and bias analyses.

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References

1. Accordini S, Calciano L, Johannessen A *et al*. A three-generation study on the association of tobacco smoking with asthma. *Int J Epidemiol* 2018;**47**:1106–17.
2. US Department of Health and Human Services. *The Health Consequences of Smoking—50 Years of Progress: A Report of the Surgeon General*. Atlanta, GA: US Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health, 2014.
3. Klingbeil EC, Hew KM, Nygaard UC, Nadeau KC. Polycyclic aromatic hydrocarbons, tobacco smoke, and epigenetic remodeling in asthma. *Immunol Res* 2014;**58**:369–73.
4. Gibbs K, Collaco JM, McGrath-Morrow SA. Impact of tobacco smoke and nicotine exposure on lung development. *Chest* 2016;**149**:552–61.
5. Singh SP, Chand HS, Langley RJ *et al*. Gestational exposure to sidestream (secondhand) cigarette smoke promotes transgenerational epigenetic transmission of exacerbated allergic asthma and bronchopulmonary dysplasia. *J Immunol* 2017;**198**:3815–22.
6. Petronis A. Epigenetics as a unifying principle in the aetiology of complex traits and diseases. *Nature* 2010;**465**:721–27.
7. Hochberg Z, Feil R, Constancia M *et al*. Child health, developmental plasticity, and epigenetic programming. *Endocr Rev* 2011;**32**:159–224.
8. Miller RL, Lawrence J. Understanding root causes of asthma: perinatal environmental exposures and epigenetic regulation. *Annals ATS* 2018;**15(Suppl 2)**:S103–08.
9. Davidson EJ, Yang IV. Role of epigenetics in the development of childhood asthma. *Curr Opin Allergy Clin Immunol* 2018;**18**:132–38.
10. Pearl J. *Causality: Models, Reasoning and Inference*. 2nd edn. New York, NY: Cambridge University Press, 2009.
11. Arah OA. The role of causal reasoning in understanding Simpson's paradox, Lord's paradox, and the suppression effect: covariate selection in the analysis of observational studies. *Emerg Themes Epidemiol* 2008;**5**:5.
12. Arah OA, Chiba Y, Greenland S. Bias formulas for external adjustment and sensitivity analysis of unmeasured confounders. *Ann Epidemiol* 2008;**18**:637–46.
13. Lash TL, Fox MP, MacLehose RF, Maldonado G, McCandless LC, Greenland S. Good practices for quantitative bias analysis. *Int J Epidemiol* 2014;**43**:1969–85.
14. VanderWeele TJ. Explanation in *Causal Inference*: *Methods* for *Mediation* and *I*nteraction. New York, NY: Oxford University Press, 2015.
15. Wang A, Arah OA. G-computation demonstration in causal mediation analysis. *Eur J Epidemiol* 2015;**30**:1119–27.
16. VanderWeele TJ. Explanation in causal inference: developments in mediation and interaction. *Int J Epidemiol* 2016;**45**:1904–08.
17. Hayes AF. *Introduction to Mediation, Moderation, and Conditional Process Analysis: A Regression-Based Approach*. 2nd edn. New York, NY: The Guilford Press, 2017.
18. Tofighi D, West SG, MacKinnon DP. Multilevel mediation analysis: the effects of omitted variables in the 1-1-1 model. *Br J Math Stat Psychol* 2013;**66**:290–307.
19. Tofighi D, Kelley K. Assessing omitted confounder bias in multilevel mediation models. *Multivariate Behav Res* 2016;**51**:86–105.
20. Preacher KJ. Multilevel SEM strategies for evaluating mediation in three-level data. *Multivariate Behav Res* 2011;**46**:691–731.
21. Kelcey B, Spybrook J, Dong N. Sample size planning for cluster-randomized interventions probing multilevel mediation. *Prev Sci* 2018; doi:10.1007/s11121-018-0921-6.
22. Rusá Š, Komárek A, Lesaffre E, Bruyneel L. Multilevel moderated mediation model with ordinal outcome. *Stat Med* 2018;**37**:1650–70.
23. Arah OA. Bias analysis for uncontrolled confounding in the health sciences. *Annu Rev Public Health* 2017;**38**:23.
24. Thompson CA, Arah OA. Selection bias modeling using observed data augmented with imputed record-level probabilities. *Ann Epidemiol* 2014;**24**:747–53.
25. Fox MP, Lash TL, Greenland S. A method to automate probabilistic sensitivity analyses of misclassified binary variables. *Int J Epidemiol* 2005;**34**:1370–76.
26. Lash TL, Fox MP, Fink AK. *Applying Quantitative Bias Analysis to Epidemiologic Data*. New York, NY: Springer Science & Business Media, 2011.
27. Greenland S. Multiple-bias modelling for analysis of observational. *J Royal Statistical Soc A* 2005;**168**:267–306.
28. Gustafson P. *Bayesian Inference for Partially Identified Models: exploring the Limits of Limited Data*. Boca Raton, FL: CRC Press, 2015.
29. Arah OA, Sudan M, Olsen J, Kheifets L. Marginal structural models, doubly robust estimation, and bias analysis in perinatal and paediatric epidemiology. *Paediatr Perinat Epidemiol* 2013;**27**:26365.