Transgenerational and intergenerational epigenetic inheritance in allergic diseases

Toril Mørkve Knudsen, BSc, MPhil,[a]* Faisal I. Rezwan, PhD,[b]* Yu Jiang, PhD,[c] Wilfried Karmaus, MD, Dr med, MPH,[c] Cecilie Svanes, MD, PhD,[d,e] and John W. Holloway, PhD[b] *Bergen, Norway, Southampton, United Kingdom, and Memphis, Tenn*

It has become clear that early life (including *in utero* exposures) is a key window of vulnerability during which environmental exposures can alter developmental trajectories and initiate allergic disease development. However, recent evidence suggests that there might be additional windows of vulnerability to environmental exposures in the parental generation before conception or even in previous generations. There is evidence suggesting that information of prior exposures can be transferred across generations, and experimental animal models suggest that such transmission can be conveyed through epigenetic mechanisms. Although the molecular mechanisms of intergenerational and transgenerationational epigenetic transmission have yet to be determined, the realization that environment before conception can alter the risks of allergic diseases has profound implications for the development of public health interventions to prevent disease. Future research in both experimental models and in multigenerational human cohorts is needed to better understand the role of intergenerational and transgenerational effects in patients with asthma and allergic disease. This will provide the knowledge basis for a new approach to efficient intervention strategies aimed at reducing the major public health challenge of these conditions. (J Allergy Clin Immunol 2018;142:765-72.)

Abbreviations used
DNAm: DNA methylation
miRNA: MicroRNA
ncRNA: Noncoding RNA
RHINESSA: Respiratory Health in Northern Europe, Spain and Australia Generation

Key words: *Transgenerational, intergenerational, multigenerational, parental, epigenetics, DNA methylation, asthma, allergy, environment*

Asthma and allergies have increased exponentially over recent decades of industrialization and urbanization. The effect and severity of these multifactorial diseases are still increasing in many low- and lower-middle income countries, particularly among younger age groups,[1-4] causing a substantial burden of disease from early childhood years. Despite major initiatives for prevention, no strategies have thus far succeeded in substantially decreasing morbidity. Asthma and allergy now constitute major common chronic inflammatory diseases worldwide and are recognized as a global public health concern.[5]

Extensive literature has addressed a large number of factors shown to be associated with asthma and allergic disease.[6,7] The more traditional risk factors include environmental toxicants,[8-10] indoor mold and dampness,[11] outdoor air pollution,[12,13] occupation,[14,15] and dietary factors.[16-18] Women's hormonal/metabolic status,[19,20] climatic factors,[21,22] tuberculosis,[23] parasitic worms,[24] and overall loss of protective factors, such as reduced exposure to infectious agents and symbiotic microorganisms,[25] are also of interest.

Epidemiologic research has increasingly acknowledged the importance of developmental origins, with early environmental exposures being key determinants for later onset of allergic disease.[26-28] In particular, early-life biodiversity[29-31] is believed to play a role in the causality of allergies. This focus on early-life development has driven a search for new approaches starting during pregnancy and early childhood to prevent allergies. However, to date, no intervention has proved effective to substantially reduce or prevent asthma and allergies.

An emerging understanding of the pathophysiologic mechanisms involved in development and persistence of allergic diseases reveals complex gene-environment interactions, with many genes having been identified in which genetic variants are associated with allergic phenotype[32-35] and interact with multiple environmental factors. However, it is clear that the inherited sequence variation associated with allergic disease across the

From [a]the Department of Clinical Medicine and [d]the Centre for International Health, Department of Global Public Health and Primary Care, University of Bergen; [b]Human Development and Health, Faculty of Medicine, University of Southampton; [c]the Division of Epidemiology, Biostatistics, and Environmental Health, School of Public Health, University of Memphis; and [e]the Department of Occupational Medicine, Haukeland University Hospital, Bergen.

*These authors contributed equally to this work.

Supported by the National Institutes of Health grants R01AI091905 and R01HL132321 (PI: Karmaus) and R01AI121226 (MPI: Zhang and Holloway), the Western Norway Health Authorities grant 912011, and the European Union (Horizon 2020, GA-633212) Ageing Lungs in European Cohorts study.

Disclosure of potential conflict of interest: F. I. Rezwan is supported by the European Union (EU). W. Karmaus receives grant support from the University of Memphis and the National Institutes of Health (NIH). C. Svanes receives grant support from the Western Norway Health Authorities and EU. J. W. Holloway receives grant support from the MRC UK, NIH, and EU. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication May 4, 2018; revised July 13, 2018; accepted for publication July 17, 2018.

Available online July 21, 2018.

Corresponding author: John W. Holloway, PhD, Human Genetics and Genomic Medicine, Duthie Building, MP808, University Hospital Southampton, Southampton SO16 6YD, United Kingdom. E-mail: j.w.holloway@soton.ac.uk.

The CrossMark symbol notifies online readers when updates have been made to the article such as errata or minor corrections

0091-6749

https://doi.org/10.1016/j.jaci.2018.07.007

genome identified to date only explains a part of the heritability of allergic disease.[36]

The epigenome refers to the information in the genome that lies "above" the DNA sequence and controls the expression of genes through mechanisms like DNA methylation (DNAm) and histone modifications. Importantly, the epigenome is in part heritable through cell division (mitosis) and is fundamental to control tissue differentiation and cellular responsiveness. The epigenome of a cell or tissue is determined by both DNA sequence and cellular or organismal environmental exposures, as well as by stochasticity. Partially stable in the course of mitosis, epigenetic information establishes a memory (or signature) of past exposures, particularly in developmental transitions. Thus, the epigenome integrates influences of the genome and developmental and environmental exposures and is increasingly recognized to play a key role in disease pathophysiology.[37]

Epigenetics has been defined by Ptashne[38] in 2007 using 3 criteria: a change in the activity of a gene (1) that does not involve a mutation, (2) that is initiated by a signal, and (3) that can result in altered disease risk in the absence of the signal that initiated its change. Classically, 4 epigenetic mechanisms have been identified: (1) DNAm, (2) histone modification, (3) chromatin remodeling, and (4) small (21- to 26-nt) noncoding RNAs (ncRNAs). There is ample evidence that DNAm fulfills all 3 criteria required to be considered an epigenetic mechanism.[39-41] Histone modifications fulfill the criteria because they have the potential to result from exogenous signals, such as cigarette smoke; alter gene activity; and are maintained through mitosis.[42-44] However, meiotic inheritance of histone modification has only been demonstrated in *Caenorhabditis elegans*.[45] DNAm usually works hand in hand with histone modifications to activate or silence genes by influencing the chromatin structure and its accessibility by transcription factors.[46]

MicroRNAs (miRNAs) are also controlled by exogenous factors and alter gene activity by either inhibiting translation or degrading mRNAs.[47,48] For instance, in human subjects, miRNAs have been demonstrated to be differentially expressed in current and never smokers and to be related to particulate matter exposure.[42,49] Currently, there is little evidence that environmentally induced miRNA expression patterns can be inherited.[50] However, because miRNAs are part of the genetic code, it is possible that DNAm can affect the activity of miRNAs and thus facilitates inheritance.

The role of epigenetic regulation in the etiology of asthma and allergy is becoming increasingly evident.[51-56] Furthermore, elucidating the epigenetic mechanisms involved in inflammation and the immune response to allergens will provide better understanding of the pathophysiology of allergic disease and a mechanistic understanding of how genes and the environment interact to determine disease susceptibility. Although the majority of studies of the epigenetics of allergic disease have focused on identifying epigenetic marks that are present before disease development (eg, in cord blood) or in patients with disease, this approach cannot explain the missing heritability (the problem in which single genetic variations are unable to explain for much of the heritability in diseases) in patients with allergic disease described above. However, the recognition that epigenetic information can be transmitted across generations (ie, through meiosis) provides a mechanism whereby epigenetics could contribute to heritability of disease and explain observations of transgenerational effects of environmental exposure on the risk of allergic diseases.[57] This review aims to summarize the evidence for transgenerational and intergenerational inheritance of allergic disease and the role of epimutations and epigenetic inheritance in patients with allergic diseases.

TRANSGENERATIONAL VERSUS INTERGENERATIONAL INHERITANCE

It is important to note that although early-life, including *in utero*, exposure to environmental factors has been shown to represent a key susceptibility window for allergic disease,[58] this does not represent true transgenerational inheritance, where epigenetic information is passed between generations. As discussed by Arshad et al,[57] there are a number of ways in which cross-generational effects can be transmitted and result in apparent transmission of disease risk between generations. Genetic inheritance across generations can explain familial resemblance in phenotypes but cannot account for alterations in disease risk as a result of environmental exposures of prior generations in the absence of continued exposure. Shared familial environment or other cultural effects can also result in similarity of disease phenotypes between generations. In addition, there is the possibility of epigenetically mediated effects to explain disease transmission or the effect of environmental factors across generations.

With regard to epigenetic effects, it is important to distinguish between intergenerational and transgenerational inheritance (Fig 1). Intergenerational effects occur when maternal environmental exposures (F0) have direct effects on the germ cells or developing fetus (including the germ line of the fetus, leading to altered phenotype of the child [F1] and possibly grandchild [F2]). On the paternal line, environmental exposures of the father can have direct effects on the germ cells that will form the child (F1). A true transgenerational effect, in which epigenetic information is transmitted across generations, can only be proved if the effect of exposure is transmitted to the F2 (on the paternal line or in a maternal line in which exposure occurred only before conception) or F3 (on the maternal line when exposure occurs during pregnancy) generation and possibly future generations in the absence of further environmental exposure or germline mutations (Fig 1).

Others have suggested that transgenerational similarity in DNAm is attributable to genetic effects by methylation quantitative trait loci[59-61]; that is, single nucleotide polymorphisms that increase the susceptibility for the methylation of specific CpGs, such as those observed at the 17q21 asthma susceptibility locus, where there is strong association between single nucleotide polymorphisms and CpG sites related to gene expression, illustrating the complex relationship between sequence variation, CpG methylation, and gene expression.[62,63]

Another mechanism through which genetic effects can cause transgenerational similarity in the epigenome is metastable epialleles. These are alleles that are variably expressed in genetically identical subjects because of epigenetic modifications established during early development and are thought to be particularly vulnerable to environmental influences,[64] such as the *Agouti* locus in mice.[65] A genetic contribution is also supported by findings that methylation and gene expression differences were smaller in monozygotic compared with dizygotic twins.[66,67] Investigation of monozygotic twins has been considered of use as a human analog of inbred animal studies.[68]

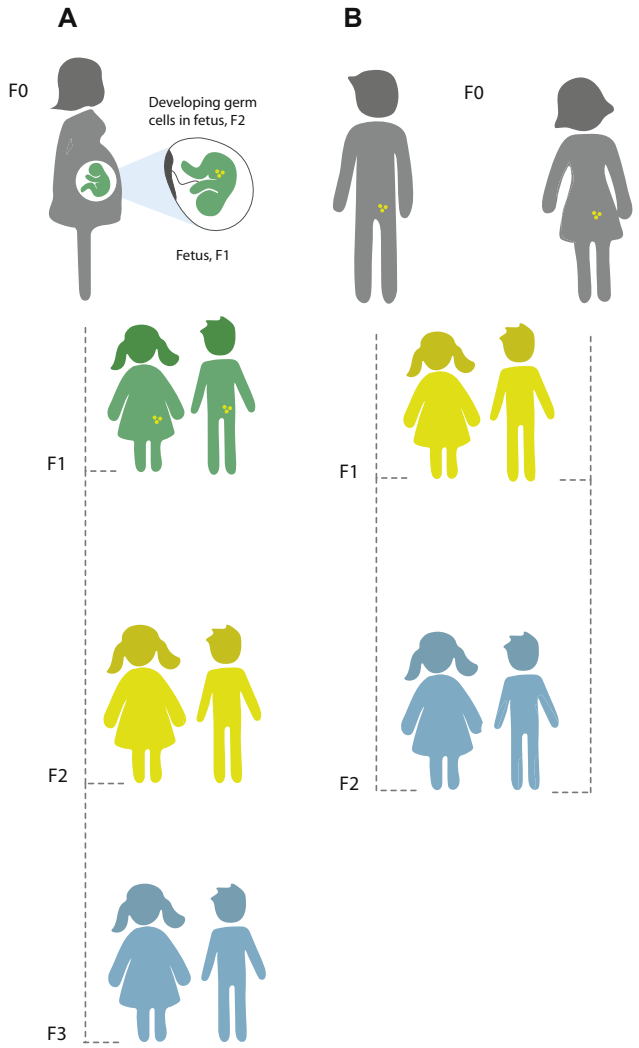

FIG 1. Principles of intergenerational and transgenerational epigenetic inheritance. **A,** If a pregnant woman *(F0)* is exposed to an environmental stressor, her son or daughter (*F1*; green), and his or her germ cells that will form the F2 generation (yellow) are also directly exposed, and this might result in intergenerational effects. The third generation (*F3*; blue) is the first generation that could represent transgenerational epigenetic inheritance. **B,** If a man or a woman (F0) and their germ cells to the F1 generation *(yellow)* are directly exposed to an environmental stressor, the F2 offspring *(blue)* is the first generation that could represent transgenerational epigenetic inheritance.

EVIDENCE FOR INTERGENERATIONAL AND TRANSGENERATIONAL INHERITANCE

A number of studies have shown that environmental exposures can lead to transgenerational inheritance of phenotypes in animal models. For example, in *Drosophila* species maternal high sugar caloric intake has been found to affect body composition and metabolism of at least 2 generations.[69] In another study exposures of mothers in early life (the larval period) to a transient high-calorie diet was found to result in a significant difference in offspring development and metabolism, and this also extended to the next generation.[70] In *C elegans* it has been found that manipulation of H3K4me3 chromatin modifiers can induce an epigenetic memory of longevity in subsequent generations[45] and that the effect of starvation-induced developmental arrest can be inherited through at least 3 generations.[71]

Evidence for transgenerational effects of environmental exposure have also been found in vertebrate models. For example, exposure of zebrafish embryos to the environmental toxin benzo [a]pyrene has been found, leading to neurobehavioral and physiologic deficits in the F2 generation.[72] In mammals, it has been demonstrated that early-life traumatic stress in the paternal line resulted in altered miRNA expression and behavioral and metabolic responses in the progeny.[73]

Exploring potential transgenerational and intergenerational epigenetic inheritance in multigenerational human studies is difficult because of the long lifecycle of human subjects, lack of data accuracy (often using the participant's recall of his or her own and previous generations' exposures and outcomes), difficulty in controlling for confounding factors, and ethical issues.[74]

Nonetheless, observational studies have suggested that transgenerational effects might exist that cannot easily be attributed to cultural and/or genetic inheritance.[75] For example, a study of the Överkalix population in northern Sweden suggested paternal transgenerational effects in human subjects. In these studies longevity and specific causes of death were linked to detailed historical records of harvests and food supply experienced by previous generations in early life.[76,77] Studies of the Dutch famine of 1944-1945 have also revealed that offspring born during the famine were smaller compared with those born the year before the famine and that they had increased risk of metabolic and cardiovascular disease in adulthood. Although differences in DNAm have been found in adult female offspring exposed to the famine *in utero* and that these offspring effects persist for 2 generations, it is not established that these differences are present in germ cells and truly reflect an epigenetic transgenerational inheritance.[78]

MOLECULAR MECHANISMS OF INTERGENERATIONAL AND TRANSGENERATIONAL INHERITANCE

Germ cells undergo extensive epigenetic reprogramming, from their earliest presence in the embryo to mature reproductive cells, and the best described reprogramming phases occur in early embryonic development and in the prepuberty period.[79] Germ cells are believed to be more susceptible to environmental influences during these reprogramming phases. However, the precise molecular mechanisms underlying transgenerational inheritance still remain unclear. It is hypothesized that transmission of information occurs through epigenetic variation in sperm, oocytes, or both sets of gametes. There are several mechanisms, such as DNAm, histone modification, or changes in ncRNA, that could play an important role in transmitting epigenetic information from one generation to the next.[79-81]

Because of its stability in stored DNA samples and comparative ease of measurement, DNAm has been the most studied epigenetic mechanism in human studies of intergenerational and transgenerational effects. However, DNAm undergoes 2 rounds of erasure in the formation of gametes and shortly after fertilization, and it is unclear whether or how memory of CpG site methylation is maintained through meiosis. Nonetheless, it has been found that the sperm epigenome can be altered by chemical compounds, such as the endocrine disruptor vinclozolin, and result in transgenerational inheritance through DNAm of induced adult-onset disease to the F3 generation.[82] In Agouti mice methyl

donor supplementation during pregnancy altered the trajectory of obesity across generations because of altered expression of the Agouti gene resulting from changes in DNAm in the offspring.[83]

Histone modification is another potential route for transgenerational inheritance. *C elegans*, although it does not exhibit DNAm-like mammals, can impart heritable epigenetic changes generated from histone modification to subsequent generations.[45]

Another possible mechanism for conveying epigenetic information between generations is ncRNAs, such as miRNA, small interfering RNA, and piwi-interacting RNA, which can potentially act as mediators of environmentally induced transgenerational inheritance. These ncRNAs show enhancer-like function and can control chromatin structure. Gapp et al[73] demonstrated that traumatic stress in early life altered mouse miRNA expression and behavioral and metabolic responses in the progeny. The phenotype of the progeny could be recapitulated by injection of sperm miRNAs into fertilized oocytes.

EPIGENETIC TRANSMISSION ACROSS GENERATIONS IN ALLERGIC DISEASE

Evidence for transmission across generations in allergic disease in animal models

Several intergenerational murine models provide evidence that preconception allergen sensitization affects the development of antigen-specific (T- and B-cell) immune responses in offspring, predisposing to development of asthma and atopy.[84-86] Mechanisms involved in regulation of allergic response have been associated with epigenetic changes of the *IL4* gene promoter,[86] as well as altered DNAm in dendritic cells.[87]

A number of studies have demonstrated adverse effects of maternal smoking and nicotine exposure on pulmonary function in offspring. *In utero* smoking has been demonstrated to affect lung growth and maturation,[88] causing alveolarization defects and decreased expression of retinoic acid signaling pathway elements,[89] as well as induced airway remodeling and lung structure changes in mice offspring.[90] Prenatal nicotine exposure has been shown to decrease forced expiratory flow rates mediated through α7 nicotinic acetylcholine receptors[91] and to affect global lung methylation levels and downregulate peroxisome proliferator-activated receptor γ expression in progeny.[92]

Maternal particle exposure has also been linked to adverse effects on lung health in offspring. Murine models have found associations between diesel exhaust particles and increased asthma susceptibility in F1 pups, with distinct methylation changes located to promoter regions of genes related to lung development, IL-4 and IFN-γ signaling,[93-95] and activation of aryl hydrocarbon receptor and oxidative stress–regulated genes.[96] Maternal exposure to specific phthalates (mono-n-butyl phthalate, a metabolite of butyl benzyl phthalate) has been shown to increase the risk for persistent airway inflammation in offspring and to induce aberrant DNAm in genes involved in T_H2 differentiation.[97]

Murine models have demonstrated that maternal exposure to microbial components and supplementation of probiotic bacteria can modulate immune responses in offspring by suppressing allergic sensitization and airway inflammation in the F1 generation.[98-100]

It has also been shown that maternal glucocorticoid-induced stress during pregnancy can increase airway inflammation and susceptibility to allergy in the offspring.[101]

Multigenerational murine models are emerging, and effects of phthalate exposures through enhanced eosinophilic airway inflammation have been reported to persist in the F2 generation.[97] It has been shown that exposure to fungi of the F0 generation was associated with decreased IgE levels and airway eosinophilia, as well as altered methylation in genes regulating T_H cells in third-generation (F_2) mice.[102] In a recent study by Gregory et al,[93] increased asthma risk after intrauterine exposure to particulate air pollution was identified up to the F3 generation. This model suggests a transgenerational effect on asthma susceptibility from exposure to environmental particles. The transgenerational murine model developed by Rehan et al[103] shows that nicotine exposure of pregnant rats is associated with increased airway resistance in F3 offspring when challenged with methacholine.

Evidence for transmission across generations in allergic disease in human subjects

The long lifecycle of human subjects makes investigating epigenetic transmission across generations in human subjects a challenge. However, recently, several studies with various solutions for obtaining multigeneration data have been published (Table I).[104-112] In different cohorts, higher asthma risk in persons whose maternal grandmother smoked has been found, even if the mother did not smoke.[104-109] In the North European RHINE study, higher asthma risk was found in persons whose paternal grandmother smoked.[110] Furthermore, this study found that father's smoking before age 15 years was associated with particularly high asthma risk in future offspring. This finding was replicated in an analysis of 2 generations in the Respiratory Health in Northern Europe, Spain and Australia Generation (RHINESSA) cohort by using advanced statistical modeling and also accounting for unmeasured confounders. Ongoing analyses of the RHINESSA cohort provides supportive evidence for a role of father's early puberty exposure in offspring health, showing lower lung function in offspring whose father smoked before age 15 years,[111] differential DNAm related to the father's smoking,[113] and higher asthma risk in offspring of fathers who became overweight before voice break.[114]

In an analysis of the European Community Respiratory Health Survey cohort, in which asthmatic/allergic disease status was measured in the parent generation at 3 time points over 20 years and allergies in offspring were reported by the parents at the third study wave, the authors found stronger associations of offspring allergies with parental asthmatic and allergic disease activity as measured before conception compared with parental status after birth.[112] This indicates that disease activity might induce changes that are transmissible to the next generation rather than a role of the shared environment, which has been termed "induced epigenetic transmission."[57]

Finally, a study of helminths and allergies in 2 generations in Norway found that fathers' *Toxocara* species exposure was associated with daughters' allergies and that mothers' *Toxocara* species exposure was associated with the sons' allergies.[24] Although parental exposure was not measured before conception, the sex-specific pattern might indicate a role for epigenetic transmission given parent-of-origin effects are seen for both genetic variation and epigenetic variation,[115,116] and risk of asthma in offspring from parental asthma has also been shown to be related to the sex of the affected parent.[117]

TABLE I. Evidence for intergenerational and transgenerational inheritance of allergic disease in human subjects

Reference	Key findings/study cohort	Exposure across generations
Accordini et al[104]	Increased asthma risk in F2 generation caused by grandmaternal smoking (F0) and paternal smoking (F1) before conception/ECRHS	Intergenerational: F0-F1-F2
Li et al[105]	Increased asthma risk in the F2 generation because of maternal (F1) and grandmaternal (F0) smoking during pregnancy/Children's Health Study in southern California (CHS)	Intergenerational: F0-F1-F2
Miller et al[106]	Increased asthma risk in F2 generation (female offspring) because of smoking by the paternal grandmother (F0) during pregnancy/Avon Longitudinal Study of Parents and children (ALSPAC)	Intergenerational: F0-F2
Magnus et al[107]	Increased asthma risk in F2 generation caused by grandmaternal (F0) smoking during pregnancy independent of the mother's smoking status/Norwegian Mother and Child Cohort Study (MoBa)	Intergenerational: F0-F2
Braback et al[108]	Increased asthma risk in F2 generation caused by smoking by the paternal grandmother (F0)/Respiratory Health In Northern Europe study (RHINE)	Intergenerational: F0-F2
Lodge et al[109]	Increased asthma risk in F2 generation caused by smoking by the paternal grandmother (F0)/RHINESSA study	Intergenerational: F0-F2
Svanes et al[110]	Increased asthma risk in F1 generation caused by paternal smoking (F0) before conception/RHINE study	Intergenerational: F0-F1
Accordini et al[111]	Lower lung function in F1 generation caused by paternal smoking (F0) before conception/ RHINESSA study	Intergenerational: F0-F1
Bertelsen et al[112]	Stronger associations of offspring (F1) allergies with parental (F0) asthmatic and allergic disease activity measured before conception compared with parental status after birth/ ECRHS study	Intergenerational: F0-F1

ECRHS, European Community Respiratory Health Survey.

Although maternal diet is increasingly recognized as a risk factor for asthma and atopy in offspring,[118] there is no current evidence to suggest that intergenerational or transgenerational effects occur in patients with allergic disease. However, maternal dietary factors, such as vitamin D and fatty acids, that have been associated with asthma risk have also been shown to be associated with DNAm changes at birth in offspring.[119,120] Further research is needed to understand whether these methylation changes lie on the casual pathway between maternal diet and allergic phenotypes in offspring.

METHODOLOGY FOR STUDYING EPIGENETIC TRANSMISSION ACROSS GENERATIONS IN PATIENTS WITH ALLERGIC DISEASE

Several approaches have been undertaken to explore transgenerational epigenetic inheritance in multigenerational human studies, including recruiting the offspring of birth cohort participants who are now reaching reproductive age, recruiting offspring/grandoffspring of adult cohorts, and using offspring recall and/or registry data to determine phenotype and/or exposures in parental generations. As mentioned before, all these approaches come with advantages and disadvantages, with compromises between prospective data collection and ease/length of cohort recruitment required. However, there are a number of multigenerational cohorts available that are already beginning to allow the assessment of intergenerational and transgenerational effects in allergic disease.[57] Although most studies have used regression models to assess the effects of prior exposure on outcome, other approaches, such as logistic regression analyses with generalized estimating equations and multilevel mediation models within a hierarchical framework,[104] are being used to account for familial clustering.

Several statistical approaches have been used to evaluate epigenetic inheritance of methylation in multigenerational cohorts. Correlation is one of the most used methods.[121,122] Strong positive correlation between parent-offspring pairs indicates a higher level of similarity of DNAm between generations. Some studies choose weighted correlation instead of Pearson correlation to minimize the variance of the correlation estimate.[123] However, observed similarity of DNAm could also be due to the fact that parent-offspring share the same environmental factors.

To distinguish environmental factors from inheritance, narrow sense heritability is defined as $h^2 = \frac{Var(A)}{Var(P)}$, where *Var(A)* is variance caused by the average effects of inheritance and *Var(P)* is total variance. Two major approaches, the path analysis model and variance of component model, are generally used to estimate heritability.[124] The component of variance can be obtained by means of ANOVA or fitting linear mixed models.[123,125] The linear mixed model is more flexible in adjusting for covariates, accounting for different types of study designs, and explicitly addressing environmental variation.[123,126]

In addition to studying epigenetic inheritance at the level of individual CpGs, transgenerational inheritance can also be evaluated for groups of CpGs that share similar pattern of DNAm transmission.[127] This approach, which incorporates unsupervised clustering into β-regression, was recently developed by Han et al[127] and was able to identify sets of CpGs that have the same/different inheritance patterns between mother-offspring and father-offspring pairs.

CONCLUSIONS

In conclusion, there is increasing evidence from both invertebrate and vertebrate experimental models that transmission of epigenetic information across generations occurs. Furthermore, experimental animal models also suggest this can lead to altered lung and immune development in response to environmental exposures in previous generations. In human subjects, studies based on historical data suggest a role for transgenerational inheritance in general, and analyses of human multigenerational data suggest intergenerational environmental effects in asthma and allergies.

Unmeasured confounding is a matter of concern in nonexperimental studies in which exposure is not randomized.[128] The only human study addressing unmeasured confounding in this context found that this error was very small[104]; still, human studies will need to be informed and complemented by careful studies in experimental models in which duration of exposure can be tightly controlled to determine precise windows of vulnerability and randomized to avoid confounding.

Careful study design will be needed to show that changes to the epigenome induced by environmental effects are passed across generations in human subjects and that the underlying epigenetic mechanisms were determined. Multigenerational cohort studies based on national and international collaboration should be established to prospectively and with a clear time order address the question on whether intergenerational and transgenerational inheritances are contributing to the risk of allergic diseases, and maximum use should be made of registry data, which can provide retrospective validated information for some generations, shortening the time frame necessary to study effects over multiple decades.

Another important area for future research is the issue of tissue specificity of DNAm. In epigenetic studies, unlike studies of DNA sequence variation, the cellular source of DNA samples is an essential consideration in study design given the extent of tissue-specific methylation.[129] The majority of studies of the epigenetics of allergic disease have used peripheral blood leukocytes because of the ease of sampling and availability of stored samples from historical cohorts, although both nasal brushings[130,131] and saliva[132] have also been used. Recently, a comparison of blood, buccal, nasal, and bronchial epithelial tissue methylation profiles has demonstrated that nasal epithelium represents the best proxy for bronchial epithelial cells.[133] However, with respect to intergenerational and transgenerational effects, it is likely that the effects on the epigenome of exposures to the developing embryo or transmitted through meiosis can manifest in multiple tissues, although this remains to be established.

If it is firmly established that intergenerational and transgenerational effects are of importance in patients with asthma and allergic disease, the potential practical consequences for public health policies are considerable. What are the time windows in which health promotion would be most efficient? A perspective on asthma and allergies might provide the knowledge basis for a new approach to efficient intervention strategies aimed at reducing the major public health challenge of asthma and allergies.

We thank the members of the Isle of Wight, ALEC, and RHINESSA research teams, who contributed to the study of multigenerational responses in allergy and asthma. J.W.H. and C.S. are members of inVIVO Planetary Health, a Group of the Worldwide Universities Network (WUN).

REFERENCES

1. Pawankar R. Allergic diseases and asthma: a global public health concern and a call to action. World Allergy Organization Journal 2014;7(1):12.
2. Bjorksten B, Clayton T, Ellwood P, Stewart A, Strachan D. Worldwide time trends for symptoms of rhinitis and conjunctivitis: phase III of the International Study of Asthma and Allergies in Childhood. Pediatr Allergy Immunol 2008; 19:110-24.
3. Pearce N, Ait-Khaled N, Beasley R, Mallol J, Keil U, Mitchell E, et al. Worldwide trends in the prevalence of asthma symptoms: phase III of the International Study of Asthma and Allergies in Childhood (ISAAC). Thorax 2007;62:758-66.
4. Asher MI, Montefort S, Bjorksten B, Lai CK, Strachan DP, Weiland SK, et al. Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: ISAAC Phases One and Three repeat multicountry cross-sectional surveys. Lancet 2006;368:733-43.
5. Beaglehole R, Bonita R, Alleyne G, Horton R, Li L, Lincoln P, et al. UN High-Level Meeting on Non-Communicable Diseases: addressing four questions. Lancet 2011;378:449-55.
6. Beasley R, Semprini A, Mitchell EA. Risk factors for asthma: is prevention possible? Lancet 2015;386:1075-85.
7. Burbank AJ, Sood AK, Kesic MJ, Peden DB, Hernandez ML. Environmental determinants of allergy and asthma in early life. J Allergy Clin Immunol 2017;140:1-12.
8. Bertelsen RJ, Longnecker MP, Lovik M, Calafat AM, Carlsen KH, London SJ, et al. Triclosan exposure and allergic sensitization in Norwegian children. Allergy 2013;68:84-91.
9. Clayton EM, Todd M, Dowd JB, Aiello AE. The impact of bisphenol A and triclosan on immune parameters in the U.S. population, NHANES 2003-2006. Environ Health Perspect 2011;119:390-6.
10. Mendell MJ, Macher JM, Kumagai K. Measured moisture in buildings and adverse health effects: a review. Indoor air 2018;28:488-99.
11. Norback D, Zock JP, Plana E, Heinrich J, Svanes C, Sunyer J, et al. Mould and dampness in dwelling places, and onset of asthma: the population-based cohort ECRHS. Occup Environ Med 2013;70:325-31.
12. Peterson B, Saxon A. Global increases in allergic respiratory disease: the possible role of diesel exhaust particles. Ann Allergy Asthma Immunol 1996;77:263-70.
13. Thurston GD, Kipen H, Annesi-Maesano I, Balmes J, Brook RD, Cromar K, et al. A joint ERS/ATS policy statement: what constitutes an adverse health effect of air pollution? An analytical framework. Eur Respir J 2017;49.
14. Svanes O, Skorge TD, Johannessen A, Bertelsen RJ, Bratveit M, Forsberg B, et al. Respiratory health in cleaners in northern europe: is susceptibility established in early life? PLoS One 2015;10:e0131959.
15. Reid PA, Reid PT. Occupational lung disease. J R Coll Physicians Edinb 2013;43: 44-8.
16. Patel S, Murray CS, Woodcock A, Simpson A, Custovic A. Dietary antioxidant intake, allergic sensitization and allergic diseases in young children. Allergy 2009;64:1766-72.
17. Weiland SK, von Mutius E, Husing A, Asher MI. Intake of trans fatty acids and prevalence of childhood asthma and allergies in Europe. ISAAC Steering Committee. Lancet 1999;353:2040-1.
18. Ellwood P, Asher MI, Bjorksten B, Burr M, Pearce N, Robertson CF. Diet and asthma, allergic rhinoconjunctivitis and atopic eczema symptom prevalence: an ecological analysis of the International Study of Asthma and Allergies in Childhood (ISAAC) data. ISAAC Phase One Study Group. Eur Respir J 2001;17: 436-43.
19. Macsali F, Real FG, Plana E, Sunyer J, Anto J, Dratva J, et al. Early age at menarche, lung function, and adult asthma. Am J Respir Crit Care Med 2011; 183:8-14.
20. Triebner K, Johannessen A, Puggini L, Benediktsdottir B, Bertelsen RJ, Bifulco E, et al. Menopause as a predictor of new-onset asthma: a longitudinal Northern European population study. J Allergy Clin Immunol 2016;137:50-7.e6.
21. Beck I, Jochner S, Gilles S, McIntyre M, Buters JT, Schmidt-Weber C, et al. High environmental ozone levels lead to enhanced allergenicity of birch pollen. PLoS One 2013;8:e80147.
22. Weiland SK, Husing A, Strachan DP, Rzehak P, Pearce N. Climate and the prevalence of symptoms of asthma, allergic rhinitis, and atopic eczema in children. Occup Environ Med 2004;61:609-15.
23. von Mutius E, Pearce N, Beasley R, Cheng S, von Ehrenstein O, Bjorksten B, et al. International patterns of tuberculosis and the prevalence of symptoms of asthma, rhinitis, and eczema. Thorax 2000;55:449-53.
24. Jogi NO, Svanes C, Siiak SP, Logan E, Holloway JW, Igland J, et al. Zoonotic helminth exposure and risk of allergic diseases: a study of two generations in Norway. Clin Exp Allergy 2018;48:66-77.
25. Okada H, Kuhn C, Feillet H, Bach JF. The 'hygiene hypothesis' for autoimmune and allergic diseases: an update. Clin Exp Immunol 2010;160:1-9.
26. Svanes C, Omenaas E, Jarvis D, Chinn S, Gulsvik A, Burney P. Parental smoking in childhood and adult obstructive lung disease: results from the European Community Respiratory Health Survey. Thorax 2004;59:295-302.
27. Duijts L. Growing large and fast: is infant growth relevant for the early origins of childhood asthma? Thorax 2016;71:1071-2.
28. Svanes C, Sunyer J, Plana E, Dharmage S, Heinrich J, Jarvis D, et al. Early life origins of chronic obstructive pulmonary disease. Thorax 2010;65:14-20.
29. von Mutius E. The microbial environment and its influence on asthma prevention in early life. J Allergy Clin Immunol 2016;137:680-9.
30. Haahtela T, Holgate S, Pawankar R, Akdis CA, Benjaponpitak S, Caraballo L, et al. The biodiversity hypothesis and allergic disease: world allergy organization position statement. World Allergy Organ J 2013;6:3.

31. Hanski I, von Hertzen L, Fyhrquist N, Koskinen K, Torppa K, Laatikainen T, et al. Environmental biodiversity, human microbiota, and allergy are interrelated. Proc Natl Acad Sci U S A 2012;109:8334-9.
32. Hirvonen A. Gene-environment interactions in chronic pulmonary diseases. Mutat Res 2009;667:132-41.
33. Douwes J, Pearce N. Asthma and the westernization 'package. Int J Epidemiol 2002;31:1098-102.
34. Schwartz DA. Gene-environment interactions and airway disease in children. Pediatrics 2009;123(suppl 3):S151-9.
35. Ober C, Vercelli D. Gene-environment interactions in human disease: nuisance or opportunity? Trends Genet 2011;27:107-15.
36. Meyers DA, Bleecker ER, Holloway JW, Holgate ST. Asthma genetics and personalised medicine. Lancet Respir Med 2014;2:405-15.
37. Feinberg AP. The key role of epigenetics in human disease prevention and mitigation. N Engl J Med 2018;378:1323-34.
38. Ptashne M. On the use of the word 'epigenetic'. Curr Biol 2007;17:R233-6.
39. Daxinger L, Whitelaw E. Understanding transgenerational epigenetic inheritance via the gametes in mammals. Nat Rev 2012;13:153-62.
40. Alegria-Torres JA, Baccarelli A, Bollati V. Epigenetics and lifestyle. Epigenomics 2011;3:267-77.
41. Jones PA. Functions of DNA methylation: islands, start sites, gene bodies and beyond. Nat Rev Genet 2012;13:484-92.
42. Lovinsky-Desir S, Miller RL. Epigenetics, asthma, and allergic diseases: a review of the latest advancements. Curr Allergy Asthma Rep 2012;12:211-20.
43. Clifford RL, John AE, Brightling CE, Knox AJ. Abnormal histone methylation is responsible for increased vascular endothelial growth factor 165a secretion from airway smooth muscle cells in asthma. J Immunol 2012;189:819-31.
44. Royce SG, Karagiannis TC. Histone deacetylases and their role in asthma. J Asthma 2012;49:121-8.
45. Greer EL, Maures TJ, Ucar D, Hauswirth AG, Mancini E, Lim JP, et al. Transgenerational epigenetic inheritance of longevity in *Caenorhabditis elegans*. Nature 2011;479:365-71.
46. Callinan PA, Feinberg AP. The emerging science of epigenomics. Hum Mol Genet 2006;15(Spec No 1):R95-101.
47. Angulo M, Lecuona E, Sznajder JI. Role of microRNAs in lung disease. Arch Bronconeumol 2012;48:325-30.
48. Su WY, Xiong H, Fang JY. Natural antisense transcripts regulate gene expression in an epigenetic manner. Biochem Biophys Res Commun 2010;396:177-81.
49. Yang IV, Schwartz DA. Epigenetic control of gene expression in the lung. Am J Respir Crit Care Med 2011;183:1295-301.
50. Buckley BA, Burkhart KB, Gu SG, Spracklin G, Kershner A, Fritz H, et al. A nuclear Argonaute promotes multigenerational epigenetic inheritance and germline immortality. Nature 2012;489:447-51.
51. Yang IV, Pedersen BS, Liu A, O'Connor GT, Teach SJ, Kattan M, et al. DNA methylation and childhood asthma in the inner city. J Allergy Clin Immunol 2015;136:69-80.
52. Xu CJ, Soderhall C, Bustamante M, Baiz N, Gruzieva O, Gehring U, et al. DNA methylation in childhood asthma: an epigenome-wide meta-analysis. Lancet Respir Med 2018;6:379-88.
53. Everson TM, Lyons G, Zhang H, Soto-Ramirez N, Lockett GA, Patil VK, et al. DNA methylation loci associated with atopy and high serum IgE: a genome-wide application of recursive random forest feature selection. Genome Med 2015;7:89.
54. Liang L, Willis-Owen SAG, Laprise C, Wong KCC, Davies GA, Hudson TJ, et al. An epigenome-wide association study of total serum immunoglobulin E concentration. Nature 2015;520:670-4.
55. Yang IV, Lozupone CA, Schwartz DA. The environment, epigenome, and asthma. J Allergy Clin Immunol 2017;140:14-23.
56. Harb H, Renz H. Update on epigenetics in allergic disease. J Allergy Clin Immunol 2015;135:15-24.
57. Arshad SH, Karmaus W, Zhang H, Holloway JW. Multigenerational cohorts in patients with asthma and allergy. J Allergy Clin Immunol 2017;139:415-21.
58. Lockett GA, Huoman J, Holloway JW. Does allergy begin in utero? Pediatr Allergy Immunol 2015;26:394-402.
59. McRae AF, Powell JE, Henders AK, Bowdler L, Hemani G, Shah S, et al. Contribution of genetic variation to transgenerational inheritance of DNA methylation. Genome Biol 2014;15:R73.
60. Gertz J, Varley KE, Reddy TE, Bowling KM, Pauli F, Parker SL, et al. Analysis of DNA methylation in a three-generation family reveals widespread genetic influence on epigenetic regulation. PLoS Genet 2011;7:e1002228.
61. Shoemaker R, Deng J, Wang W, Zhang K. Allele-specific methylation is prevalent and is contributed by CpG-SNPs in the human genome. Genome Res 2010;20:883-9.
62. Acevedo N, Reinius LE, Greco D, Gref A, Orsmark-Pietras C, Persson H, et al. Risk of childhood asthma is associated with CpG-site polymorphisms, regional DNA methylation and mRNA levels at the GSDMB/ORMDL3 locus. Hum Mol Genet 2015;24:875-90.
63. Kothari PH, Qiu W, Croteau-Chonka DC, Martinez FD, Liu AH, Lemanske RF Jr, et al. Role of local CpG DNA methylation in mediating the 17q21 asthma susceptibility gasdermin B (GSDMB)/ORMDL sphingolipid biosynthesis regulator 3 (ORMDL3) expression quantitative trait locus. J Allergy Clin Immunol 2018;141:2282-6.e6.
64. Dolinoy DC, Das R, Weidman JR, Jirtle RL. Metastable epialleles, imprinting, and the fetal origins of adult diseases. Pediatr Res 2007;61:30R-7R.
65. Blewitt M, Whitelaw E. The use of mouse models to study epigenetics. Cold Spring Harb Perspect Biol 2013;5:a017939.
66. Loke YJ, Novakovic B, Ollikainen M, Wallace EM, Umstad MP, Permezel M, et al. The Peri/postnatal Epigenetic Twins Study (PETS). Twin Res Hum Genet 2013;16:13-20.
67. Ollikainen M, Smith KR, Joo EJ, Ng HK, Andronikos R, Novakovic B, et al. DNA methylation analysis of multiple tissues from newborn twins reveals both genetic and intrauterine components to variation in the human neonatal epigenome. Hum Mol Genet 2010;19:4176-88.
68. Van Baak TE, Coarfa C, Dugue PA, Fiorito G, Laritsky E, Baker MS, et al. Epigenetic supersimilarity of monozygotic twin pairs. Genome Biol 2018;19:2.
69. Buescher JL, Musselman LP, Wilson CA, Lang T, Keleher M, Baranski TJ, et al. Evidence for transgenerational metabolic programming in *Drosophila*. Dis Model Mech 2013;6:1123-32.
70. Matzkin LM, Johnson S, Paight C, Markow TA. Preadult parental diet affects offspring development and metabolism in *Drosophila melanogaster*. PLoS One 2013;8:e59530.
71. Rechavi O, Houri-Ze'evi L, Anava S, Goh WSS, Kerk SY, Hannon GJ, et al. Starvation-induced transgenerational inheritance of small RNAs in *C. elegans*. Cell 2014;158:277-87.
72. Knecht AL, Truong L, Marvel SW, Reif DM, Garcia A, Lu C, et al. Transgenerational inheritance of neurobehavioral and physiological deficits from developmental exposure to benzo[a]pyrene in zebrafish. Toxicol Appl Pharmacol 2017;329:148-57.
73. Gapp K, Jawaid A, Sarkies P, Bohacek J, Pelczar P, Prados J, et al. Implication of sperm RNAs in transgenerational inheritance of the effects of early trauma in mice. Nat Neurosci 2014;17:667-9.
74. Rothstein MA, Cai Y, Marchant GE. The ghost in our genes: legal and ethical implications of epigenetics. Health Matrix Clevel 2009;19:1-62.
75. Pembrey M, Saffery R, Bygren LO. Network in Epigenetic Epidemiology. Human transgenerational responses to early-life experience: potential impact on development, health and biomedical research. J Med Genet 2014;51:563-72.
76. Pembrey ME, Bygren LO, Kaati G, Edvinsson S, Northstone K, Sjostrom M, et al. Sex-specific, male-line transgenerational responses in humans. Eur J Hum Genet 2006;14:159-66.
77. Kaati G, Bygren LO, Pembrey M, Sjostrom M. Transgenerational response to nutrition, early life circumstances and longevity. Eur J Hum Genet 2007;15:784-90.
78. Veenendaal MV, Painter RC, de Rooij SR, Bossuyt PM, van der Post JA, Gluckman PD, et al. Transgenerational effects of prenatal exposure to the 1944-45 Dutch famine. BJOG 2013;120:548-53.
79. Wu H, Hauser R, Krawetz SA, Pilsner JR. Environmental susceptibility of the sperm epigenome during windows of male germ cell development. Curr Environ Health Rep 2015;2:356-66.
80. Heard E, Martienssen RA. Transgenerational epigenetic inheritance: myths and mechanisms. Cell 2014;157:95-109.
81. Sales VM, Ferguson-Smith AC, Patti ME. Epigenetic mechanisms of transmission of metabolic disease across generations. Cell Metab 2017;25:559-71.
82. Guerrero-Bosagna C, Covert TR, Haque MM, Settles M, Nilsson EE, Anway MD, et al. Epigenetic transgenerational inheritance of vinclozolin induced mouse adult onset disease and associated sperm epigenome biomarkers. Reprod Toxicol 2012;34:694-707.
83. Wolff GL, Kodell RL, Moore SR, Cooney CA. Maternal epigenetics and methyl supplements affect agouti gene expression in Avy/a mice. FASEB J 1998;12:949-57.
84. Victor JR, Muniz BP, Fusaro AE, de Brito CA, Taniguchi EF, Duarte AJ, et al. Maternal immunization with ovalbumin prevents neonatal allergy development and up-regulates inhibitory receptor Fc gamma RIIB expression on B cells. BMC Immunol 2010;11:11.
85. Herz U, Joachim R, Ahrens B, Scheffold A, Radbruch A, Renz H. Prenatal sensitization in a mouse model. Am J Respir Crit Care Med 2000;162:S62-5.
86. Song Y, Liu C, Hui Y, Srivastava K, Zhou Z, Chen J, et al. Maternal allergy increases susceptibility to offspring allergy in association with TH2-biased epigenetic alterations in a mouse model of peanut allergy. J Allergy Clin Immunol 2014;134:1339-45.e7.

87. Fedulov AV, Kobzik L. Allergy risk is mediated by dendritic cells with congenital epigenetic changes. Am J Respir Cell Mol Biol 2011;44:285-92.
88. Larcombe AN, Foong RE, Berry LJ, Zosky GR, Sly PD. In utero cigarette smoke exposure impairs somatic and lung growth in BALB/c mice. Eur Respir J 2011; 38:932-8.
89. Manoli SE, Smith LA, Vyhlidal CA, An CH, Porrata Y, Cardoso WV, et al. Maternal smoking and the retinoid pathway in the developing lung. Respir Res 2012;13:42.
90. Blacquiere MJ, Timens W, Melgert BN, Geerlings M, Postma DS, Hylkema MN. Maternal smoking during pregnancy induces airway remodelling in mice offspring. Eur Respir J 2009;33:1133-40.
91. Wongtrakool C, Wang N, Hyde DM, Roman J, Spindel ER. Prenatal nicotine exposure alters lung function and airway geometry through alpha7 nicotinic receptors. Am J Respir Cell Mol Biol 2012;46:695-702.
92. Rehan VK, Liu J, Naeem E, Tian J, Sakurai R, Kwong K, et al. Perinatal nicotine exposure induces asthma in second generation offspring. BMC Med 2012;10:129.
93. Gregory DJ, Kobzik L, Yang Z, McGuire CC, Fedulov AV. Transgenerational transmission of asthma risk after exposure to environmental particles during pregnancy. Am J Physiol Lung Cell Mol Physiol 2017;313:L395-405.
94. Fedulov AV, Leme A, Yang Z, Dahl M, Lim R, Mariani TJ, et al. Pulmonary exposure to particles during pregnancy causes increased neonatal asthma susceptibility. Am J Respir Cell Mol Biol 2008;38:57-67.
95. Liu J, Ballaney M, Al-alem U, Quan C, Jin X, Perera F, et al. Combined inhaled diesel exhaust particles and allergen exposure alter methylation of T helper genes and IgE production in vivo. Toxicol Sci 2008;102:76-81.
96. Manners S, Alam R, Schwartz DA, Gorska MM. A mouse model links asthma susceptibility to prenatal exposure to diesel exhaust. J Allergy Clin Immunol 2014;134:63-72.
97. Jahreis S, Trump S, Bauer M, Bauer T, Thurmann L, Feltens R, et al. Maternal phthalate exposure promotes allergic airway inflammation over 2 generations through epigenetic modifications. J Allergy Clin Immunol 2018;141:741-53.
98. Blumer N, Herz U, Wegmann M, Renz H. Prenatal lipopolysaccharide-exposure prevents allergic sensitization and airway inflammation, but not airway responsiveness in a murine model of experimental asthma. Clin Exp Allergy 2005;35: 397-402.
99. Blumer N, Sel S, Virna S, Patrascan CC, Zimmermann S, Herz U, et al. Perinatal maternal application of *Lactobacillus rhamnosus* GG suppresses allergic airway inflammation in mouse offspring. Clin Exp Allergy 2007;37:348-57.
100. Brand S, Teich R, Dicke T, Harb H, Yildirim AO, Tost J, et al. Epigenetic regulation in murine offspring as a novel mechanism for transmaternal asthma protection induced by microbes. J Allergy Clin Immunol 2011;128:618-25, e1-7.
101. Lim R, Fedulov AV, Kobzik L. Maternal stress during pregnancy increases neonatal allergy susceptibility: role of glucocorticoids. Am J Physiol Lung Cell Mol Physiol 2014;307:L141-8.
102. Niedzwiecki M, Zhu H, Corson L, Grunig G, Factor PH, Chu S, et al. Prenatal exposure to allergen, DNA methylation, and allergy in grandoffspring mice. Allergy 2012;67:904-10.
103. Rehan VK, Liu J, Sakurai R, Torday JS. Perinatal nicotine-induced transgenerational asthma. Am J Physiol Lung Cell Mol Physiol 2013;305:L501-7.
104. Accordini S, Calciano L, Johannessen A, Portas L, Benediktsdottir B, Bertelsen RJ, et al. A three-generation study on the association of tobacco smoking with asthma. Int J Epidemiol 2018 [Epub ahead of print].
105. Li YF, Langholz B, Salam MT, Gilliland FD. Maternal and grandmaternal smoking patterns are associated with early childhood asthma. Chest 2005; 127:1232-41.
106. Miller LL, Henderson J, Northstone K, Pembrey M, Golding J. Do grandmaternal smoking patterns influence the etiology of childhood asthma? Chest 2014;145: 1213-8.
107. Magnus MC, Haberg SE, Karlstad O, Nafstad P, London SJ, Nystad W. Grandmother's smoking when pregnant with the mother and asthma in the grandchild: the Norwegian Mother and Child Cohort Study. Thorax 2015;70:237-43.
108. Braback L, Lodge CJ, Lowe AJ, Dharmage SC, Olsson D, Forsberg B. Childhood asthma and smoking exposures before conception—a three-generational cohort study. Pediatr Allergy Immunol 2018;29:361-8.
109. Lodge CJ, Braback L, Lowe AJ, Dharmage SC, Olsson D, Forsberg B. Grandmaternal smoking increases asthma risk in grandchildren: a nationwide Swedish cohort. Clin Exp Allergy 2018;48:167-74.
110. Svanes C, Koplin J, Skulstad SM, Johannessen A, Bertelsen RJ, Benediktsdottir B, et al. Father's environment before conception and asthma risk in his children: a multi-generation analysis of the Respiratory Health In Northern Europe study. Int J Epidemiol 2017;46:235-45.
111. Accordini S, Johannessen A, Calciano L, Jogi R, Martinez-Moratalla Rovira J, Benediktsdottir B, et al. Three-generation effects of tobacco smoking on lung function within the paternal line. Eur Respir J 2017;50(suppl 61): PA1178.
112. Bertelsen RJ, Rava M, Carsin AE, Accordini S, Benediktsdottir B, Dratva J, et al. Clinical markers of asthma and IgE assessed in parents before conception predict asthma and hayfever in the offspring. Clin Exp Allergy 2017;47:627-38.
113. Mørkve Knudsen T, Rezwan F, Skulstad SM, Bertelsen RJ, Gomez Real F, Probst-Hensch N, et al. Late breaking abstract—epigenome-wide association of father's smoking on offspring DNA methylation. Eur Respir J 2017; 50(suppl 61):OA2948.
114. Johannessen A, Calciano L, Lonnebotn M, Bertelsen RJ, Braback L, Holm M, et al. Late breaking abstract—fathers' overweight and offspring asthma—an intergenerational perspective. Eur Respir J 2017;50(suppl 61):PA2615.
115. Martino D, Prescott S. Epigenetics and prenatal influences on asthma and allergic airways disease. Chest 2011;139:640-7.
116. Moffatt MF, Cookson WO. The genetics of asthma. Maternal effects in atopic disease. Clin Exp Allergy 1998;28(suppl 1):56-61.
117. Arshad SH, Karmaus W, Raza A, Kurukulaaratchy RJ, Matthews SM, Holloway JW, et al. The effect of parental allergy on childhood allergic diseases depends on the sex of the child. J Allergy Clin Immunol 2012;130:427-34.
118. Lee-Sarwar K, Litonjua AA. As you eat it: effects of prenatal nutrition on asthma. J Allergy Clin Immunol Pract 2018;6:711-8.
119. Suderman M, Stene LC, Bohlin J, Page CM, Holvik K, Parr CL, et al. 25-Hydroxyvitamin D in pregnancy and genome wide cord blood DNA methylation in two pregnancy cohorts (MoBa and ALSPAC). J Steroid Biochem Mol Biol 2016;159: 102-9.
120. van Dijk SJ, Zhou J, Peters TJ, Buckley M, Sutcliffe B, Oytam Y, et al. Effect of prenatal DHA supplementation on the infant epigenome: results from a randomized controlled trial. Clin Epigenet 2016;8:114.
121. Kile ML, Baccarelli A, Tarantini L, Hoffman E, Wright RO, Christiani DC. Correlation of global and gene-specific DNA methylation in maternal-infant pairs. PLoS One 2010;5:e13730.
122. Cullati S, Courvoisier DS, Gayet-Ageron A, Haller G, Irion O, Agoritsas T, et al. Patient enrollment and logistical problems top the list of difficulties in clinical research: a cross-sectional survey. BMC Med Res Methodol 2016;16:50.
123. Day K, Waite LL, Alonso A, Irvin MR, Zhi D, Thibeault KS, et al. Heritable DNA methylation in CD4+ cells among complex families displays genetic and non-genetic effects. PLoS One 2016;11:e0165488.
124. McArdle JJ, Prescott CA. Mixed-effects variance components models for biometric family analyses. Behav Genet 2005;35:631-52.
125. Rowlatt A, Hernandez-Suarez G, Sanabria-Salas MC, Serrano-Lopez M, Rawlik K, Hernandez-Illan E, et al. The heritability and patterns of DNA methylation in normal human colorectum. Hum Mol Genet 2016;25:2600-11.
126. Heckerman D, Gurdasani D, Kadie C, Pomilla C, Carstensen T, Martin H, et al. Linear mixed model for heritability estimation that explicitly addresses environmental variation. Proc Natl Acad Sci U S A 2016;113:7377-82.
127. Han S, Zhang H, Lockett GA, Mukherjee N, Holloway JW, Karmaus W. Identifying heterogeneous transgenerational DNA methylation sites via clustering in beta regression. Ann Appl Stat 2015;9:2052-72.
128. Uddin MJ, Groenwold RH, Ali MS, de Boer A, Roes KC, Chowdhury MA, et al. Methods to control for unmeasured confounding in pharmacoepidemiology: an overview. Int J Clin Pharm 2016;38:714-23.
129. Byun HM, Siegmund KD, Pan F, Weisenberger DJ, Kanel G, Laird PW, et al. Epigenetic profiling of somatic tissues from human autopsy specimens identifies tissue- and individual-specific DNA methylation patterns. Hum Mol Genet 2009; 18:4808-17.
130. Somineni HK, Zhang X, Biagini Myers JM, Kovacic MB, Ulm A, Jurcak N, et al. Ten-eleven translocation 1 (TET1) methylation is associated with childhood asthma and traffic-related air pollution. J Allergy Clin Immunol 2016;137: 797-805.e5.
131. Yang IV, Pedersen BS, Liu AH, O'Connor GT, Pillai D, Kattan M, et al. The nasal methylome and childhood atopic asthma. J Allergy Clin Immunol 2017;139: 1478-88.
132. Langie SAS, Moisse M, Szarc Vel Szic K, Van Der Plas E, Koppen G, De Prins S, et al. GLI2 promoter hypermethylation in saliva of children with a respiratory allergy. Clin Epigenet 2018;10:50.
133. Brugha R, Lowe R, Henderson AJ, Holloway JW, Rakyan V, Wozniak E, et al. DNA methylation profiles between airway epithelium and proxy tissues in children. Acta Paediatr 2017;106:2011-6.