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 transgenerational 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.)

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, 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 chronic inflammatory diseases worldwide and are recognized as a global public health concern.

Extensive literature has addressed a large number of factors shown to be associated with asthma and allergic disease. The more traditional risk factors include environmental toxicants, indoor mold and dampness, outdoor air pollution, occupational, and dietary factors. Women’s hormonal/metabolic status, climatic factors, tuberculosis, parasitic worms, and overall loss of protective factors, such as reduced exposure to infectious agents and symbiotic microorganisms, are also of interest.

Epidemiologic research has increasingly acknowledged the importance of development origins, with early environmental exposures being key determinants for later onset of allergic disease. In particular, early-life biodiversity 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 and interact with multiple environmental factors. However, it is clear that the inherited sequence variation associated with allergic disease across the
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.

Epigenetics has been defined by Ptashne 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. 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. However, meiotic inheritance of histone modification has only been demonstrated in Caenorhabditis elegans. DNA methylation usually works hand in hand with histone modifications to activate or silence genes by influencing the chromatin structure and its accessibility by transcription factors.

MicroRNAs (miRNAs) are also controlled by exogenous factors and alter gene activity by either inhibiting translation or degrading mRNAs. 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. Currently, there is little evidence that environmentally induced miRNA expression patterns can be inherited. 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. 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. 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, this does not represent true transgenerational inheritance, where epigenetic information is passed between generations. As discussed by Arshad et al, 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; 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.

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, such as the Agouti locus in mice. A genetic contribution is also supported by findings that methylation and gene expression differences were smaller in monozygotic compared with dizygotic twins. Investigation of monozygotic twins has been considered of use as a human analog of inbred animal studies.
and that the effect of starvation-induced developmental arrest can be inherited through at least 3 generations.

Evidence for transgenerational effects of environmental exposure have also been found in vertebrate models. For example, exposure of zebrafish embryos to the environmental toxin benzopyrene has been found, leading to neurobehavioral and physiologic deficits in the F2 generation. 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.

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.

Nonetheless, observational studies have suggested that transgenerational effects might exist that cannot easily be attributed to cultural and/or genetic inheritance. For example, a study of the Overkalix 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. 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 DNA methylation 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.

**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. 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.

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. 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.

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.

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 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. Mechanisms involved in regulation of allergic response have been associated with epigenetic changes of the *IL4* gene promoter, as well as altered DNAm in dendritic cells.

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, causing alveolarization defects and decreased expression of retinoic acid signaling pathway elements, as well as induced airway remodeling and lung structure changes in mice offspring. Prenatal nicotine exposure has been shown to decrease forced expiratory flow rates mediated through α7 nicotinic acetylcholine receptors and to affect global lung methylation levels and downregulate peroxisome proliferator-activated receptor γ expression in progeny.

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, and activation of aryl hydrocarbon receptor and oxidative stress–regulated genes. 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 TIL2 differentiation.

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.

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

Multigenerational murine models are emerging, and effects of phthalate exposures through enhanced eosinophilic airway inflammation have been reported to persist in the F2 generation. 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<sub>g</sub>* cells in third-generation (F3) mice. In a recent study by Gregory et al, 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 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). In different cohorts, higher asthma risk in persons whose maternal grandmother smoked has been found, even if the mother did not smoke. The North European RHINESSA study, higher asthma risk was found in persons whose paternal grandmother smoked. 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, differential DNAm related to the father’s smoking, and higher asthma risk in offspring of fathers who became overweight before voice break.

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. 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.”

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. 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, and risk of asthma in offspring from parental asthma has also been shown to be related to the sex of the affected parent.
Although maternal diet is increasingly recognized as a risk factor for asthma and atopy in offspring, 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 DNA methylation changes at birth in offspring. Further research is needed to understand whether these methylation changes lie on the causal 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. 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, 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. Strong positive correlation between parent-offspring pairs indicates a higher level of similarity of DNA methylation between generations. Some studies choose weighted correlation instead of Pearson correlation to minimize the variance of the correlation estimate. However, observed similarity of DNA methylation 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 $h^2 = \frac{\text{Var}(A)}{\text{Var}(P)}$, where $\text{Var}(A)$ is the variance caused by the average effects of inheritance and $\text{Var}(P)$ is total variance. Two major approaches, the path analysis model and variance of component model, are generally used to estimate heritability. The component of variance can be obtained by means of ANOVA or fitting linear mixed models. The linear mixed model is more flexible in adjusting for covariates, accounting for different types of study designs, and explicitly addressing environmental variation.

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 DNA methylation. This approach, which incorporates unsupervised clustering into β-regression, was recently developed by Han et al. 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. The only human study addressing unmeasured confounding in this context found that this error was very small; 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. 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 and saliva 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. 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 allergic disease, the potential practical consequences for public health policies are considerable. What are the time windows in allergic disease, the potential practical consequences for public health policies are considerable.

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