

Multigenerational cohorts in patients with asthma and allergy



S. Hasan Arshad, DM,^{a,b} Wilfried Karmaus, Drmed,^c Hongmei Zhang, PhD,^c and John W. Holloway, PhD^{b,d}
and Southampton, United Kingdom, and Memphis, Tenn

Isle of Wight

Recent observations that disease risk can be transmitted across generations without the need for direct exposure of the child to the index environmental insult has sparked interest in transgenerational inheritance. Epigenetics describes processes that modify gene expression without a change in the nucleotide sequence. Epigenetic processes can be induced in response to environmental exposures, can influence disease risk, and might explain these multigenerational effects. In experimental models a number of epigenetic mechanisms have been identified that could mediate vertical transmission of epigenetic inheritance. However, relevance of these findings to human disease is not yet clear. An alternative model is one in which transgenerational inheritance of disease risk requires the presence of exposure-related diseases in the mother during pregnancy (termed induced epigenetic transmission model). A number of cross-sectional studies have investigated multigenerational effects in allergy and asthma. However, given the early-life origins of asthma and allergy, birth cohort studies are ideal to investigate the effect of genetic predisposition, epigenetics, and environmental exposures, avoiding pitfalls, such as recall bias and confounding by ongoing exposures, disease, and treatment. The well-characterized 3 generations of the Isle of Wight cohort include 2 consecutive birth cohorts, providing longitudinal data that can be studied for epigenetic transfer of information, such

as the effect of grand parental smoking or exposure to other toxic compounds. Further large multigenerational birth cohorts are needed to establish the clinical relevance of this phenomenon and differentiate between vertical and induced transmission models, which might influence future preventive strategies. (J Allergy Clin Immunol 2017;139:415-21.)

Key words: Cohort, multigenerational, epigenetics, asthma, allergy, DNA methylation

Atopy, which is defined as the genetic susceptibility to produce IgE in response to allergen exposure, underlies allergic diseases, including asthma, eczema, rhinitis, and food allergy. The majority of the US population is affected by atopy.¹ Both shared genetics and environment explain the risk of allergic diseases transferred from one generation to the next. However, disentangling genetic from environmental effects is a major challenge for most common chronic conditions. Considerable evidence supports the early-life origins of allergic diseases, even as early as the gestational period, a concept known as fetal or developmental programming. Hence cohorts starting during pregnancy can assess the early developmental origins of health without confounding by environment or treatment in later life. More intriguing is the observations that not only maternal exposures, such as smoking, but also grand-maternal and paternal line environmental exposures are associated with the development of wheeze and asthma. These multigenerational effects can be explained by epigenetic mechanisms, which are thought to mediate developmental programming across generations. Without proper knowledge of epigenetic transfer across generations, we cannot decipher the effects of pregnancy-related exposures from the existing parental effects. Multigenerational cohorts are key to investigating these mechanisms.

MULTIGENERATIONAL COHORTS

In addition to parental genetic effects, environmental exposures of parents and grandparents have been increasingly recognized as determining individual health, even later in life. Maternal exposures, especially during the prenatal period, can have a direct effect through placental transfer of nutrients, metabolites, and environmental pollutants. However, a number of studies have shown how paternal and grandparental characteristics and environmental exposures affect children's and grandchildren's growth and development.²⁻⁵ Thus developmental programming can be transmitted across generations where the index child was not exposed to the environment that triggered the change. Hence the risk to the subject's health has to be

From ^athe David Hide Asthma and Allergy Research Centre, Isle of Wight; ^bClinical and Experimental Sciences, Faculty of Medicine, University of Southampton; ^cthe Division of Epidemiology, Biostatistics and Environmental Health, School of Public Health, University of Memphis; and ^dHuman Development and Health, Faculty of Medicine, University of Southampton, Southampton General Hospital.

The Isle of Wight birth cohort assessments have been supported by the National Institutes of Health USA (grant nos. R01 HL082925, R01 AI091905, R01 AI121226, and R01 HL132321) and Asthma UK (grant no. 364). S.H.A., W.K., H.Z., and J.W.H. and ongoing studies in the Isle of Wight cohorts are currently supported by the National Institutes of Health (R01AI121226, and R01AI091905, and 1R01HL132321). J.W.H. acknowledges support from the Ageing Lungs in European Cohorts Study (www.alecstudy.org), which has been funded by the European Union's Horizon 2020 Research and Innovation programme under grant agreement no. 633212.

Disclosure of potential conflict of interest: S. H. Arshad receives grant support from the National Institutes of Health (NIH), National Institute of Health Research UK, and Medical Research Council and serves on the board for Allergy Therapeutics. W. Karmaus receives grant support from the University of Memphis. H. Zhang receives research support from the NIH. J. W. Holloway receives research support from the NIH and European Union.

Received for publication October 13, 2016; revised November 30, 2016; accepted for publication December 2, 2016.

Corresponding author: S. Hasan Arshad, DM, MP:810, South Block, Southampton General Hospital, Tremona Road, Southampton, United Kingdom. E-mail: sha@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/\$36.00

© 2016 American Academy of Allergy, Asthma & Immunology

<http://dx.doi.org/10.1016/j.jaci.2016.12.002>

Abbreviations used

DNA-M: DNA methylation

ISAAC: International Study of Asthma and Allergies in Childhood

investigated with the knowledge of cross-generational influences. The effects on grandchildren of grandparental characteristics or environmental exposures can either be intergenerational or transgenerational in nature (Table I). In intergenerational effects maternal exposure (F0) has direct effects on the developing fetus and potentially on the germline of the fetus, leading to altered phenotype of the child (F1) and grandchild (F2). Evidence for transgenerational inheritance is rarer but has been observed both in animal models⁶⁻⁹ and human subjects.^{5,10-12}

Cross-generational studies can reveal intriguing findings. For example, the Hispanic health paradox (Hispanic immigrants tend to have better health outcomes than their US-born and white counterparts) with regard to asthma and allergy was studied by using a cross-sectional design encompassing 4 generations. This showed that asthma and allergy rates were low in the immigrant generation but increased with each subsequent generation born in the United States, with the healthy immigrant advantage lasting for 2 to 3 generations for allergies and asthma.¹³ The Respiratory Health In Northern Europe, Spain and Australia (RHINESSA) study (www.rhinessa.net) is recruiting the children and parents of participants in large cross-sectional studies (Respiratory Health in Northern Europe [RHINE]¹⁴ and the European Community Respiratory Health Survey [ECRHS]¹⁵) in 7 countries. These studies have investigated lung health, allergies, and associated diseases over the last 20 years. However, in epidemiologic studies a prospective cohort design is preferable because it avoids selection and recall biases. One of the largest longitudinal multigenerational studies is composed of the French E3N and E4N studies. The E3N study enrolled 100,000 women between 40 and 65 years of age since 1990, focusing on a range of diseases, such as cancer, diabetes, obesity, and asthma.¹⁶ Their children and grandchildren have recently been recruited in a multigenerational cohort study (E4N) to investigate exposures to environmental factors associated with disease risk in an intergenerational and transgenerational approach.

Birth cohort studies are ideal to investigate the effect of genetic predisposition, epigenetics, and exposures during early life on the occurrence and progression of asthma and allergies. The advantage is that data and samples are available before disease development, which could help ascertain causation and help identify predictive biomarkers. The Lifeways cross-generation cohort study recruited approximately 1000 children and their parents, as well as determining information on health status, dietary intake, and adult chronic disease in grandparents of both lineages. Child health outcomes include wheeze and asthma, as well as obesity.¹⁷

The ACROSSOLAR study is recruiting children born to approximately 2000 participants of the International Study of Asthma and Allergies in Childhood (ISAAC) in Germany.¹⁸ Information is collected regarding pregnancy conditions and asthma and allergic health status of the child up to primary school age. Thus information is available for 3 generations (ISAAC participants, their parents, and their children). In addition, DNA for epigenetic analysis is collected.

Likewise, the Avon Longitudinal Study of Parents and Children (ALSPAC) birth cohort¹⁹ has recently started enrolling both the grandparents and offspring of the original birth cohort. Table II^{16-18,20} provides a summary of multigenerational cohort studies with a focus on asthma and allergy.

Isle of Wight third-generation cohort

The Isle of Wight cohort includes 3 generations: the first generation (F0; grandparents, *n* = 1536); the second generation (F1, *n* = 1456), the original birth cohort; and the third generation (F2, *n* = 403), the children of the original birth cohort participants (Fig 1). The Isle of Wight is an island just off the southern coast of England with a resident population of 138,000. The Isle of Wight cohort is largely of white descent (98%). The first generation (grandparents) was enrolled at the time of childbirth, and data and samples were collected to assess asthma and allergic status. The second generation (the Isle of Wight cohort) was recruited at birth and assessed extensively and repeatedly for asthma and allergies up to the age of 27 years. Since 2010, third-generation children (born to ≥ 1 second-generation parent, either mother or father) have been enrolled.

The overall objective of the study is to gain a better understanding of the natural history of asthma, eczema, allergic diseases, and obesity during the life course over 3 generations. Hence allergic disease and exposure were extensively characterized at different ages from infancy to 27 years of age. Furthermore, the study aims to identify environmental, genetic, and epigenetic risk factors and their transition (behavioral, genetic, epigenetic, and skin and gut microbiome) over generations. An important question addresses the role of *in utero* exposure versus genetic inheritance. Hence multiple samples have been collected during pregnancy and in subsequent years from the F0, F1, and F2 generations. Importantly, there has been very low attrition, with participation of 93% of the original cohort at 10 years and 90% at 18 years. This unique population-based study integrates 2 consecutive birth cohorts providing data to construct intergenerational trajectories of wheezing, asthma, eczema, rhinitis, allergic sensitization, and lung function with an opportunity to study the role of *in utero* conditions, genetics, and epigenetics in transferring health risk over generations.²¹ For instance, the Isle of Wight multigenerational study recently showed that by using “no smoking by either mother or grandmother” as a control, the odds of wheeze in early childhood was higher (2.6; 95% CI, 0.9-7.1) when both the mother and grandmother smoked during pregnancy compared with when only the mother (0.9; 95% CI, 0.4-2.3) or grandmother (0.9; 95% CI, 0.3-2.4) smoked.²⁰ Whether these effects across generations are transmitted through epigenetic mechanisms is being investigated.

In a mouse model the transgenerational transmission of the asthma phenotype to F3 offspring (fourth generation) after perinatal nicotine exposure of F0 has been demonstrated and presumed to be due to transgenerational transfer of epigenetic information across the germline.²² However, multigenerational cohort studies are essential to confirm these effects in human subjects. The Isle of Wight study, which conducted a genome-wide assessment, indicated that in this unselected population DNA methylation (DNA-M) was transmitted from parents to offspring only at a small portion (approximately 1%) of all the cytosine-guanine (CpG) sites. Among these 1% CpG sites, most

TABLE I. Definitions commonly used when describing transfer of information across generations

Epigenetic multigenerational inheritance	Transmission of epigenetic information through the germline across generations in the absence of any direct environmental exposure or genetic manipulation
Intergenerational inheritance	Maternal exposure (F0) has direct effects on the developing fetus and potentially on the germline of the fetus, leading to altered phenotype of the child (F1) and grandchild (F2)
Transgenerational inheritance	Effect on subsequent generation persists in absence of the possibility of direct environmental exposure, such as effects persisting to the greatgrandchild (F3) on the maternal line or effects of prepubertal paternal or grandpaternal exposures in the male line

TABLE II. Multigenerational cohorts in the field of atopic diseases

Author/study/reference	Population studied	Variables	Main outcomes
French E3N/E4N Study ¹⁶	France; n = 98,995 (started 1990)*	BMI, education, exercise, smoking	Cancer, diabetes, bone health, asthma
Lifeways cross-generation cohort study ¹⁷	Ireland; n = 1,128 (families [started 2001]) Third generation; n = 1,094 (2001-2013)	Diet, sex smoking, age, breast-feeding, birth weight	BMI, wheeze, asthma
ACROSSOLAR study ¹⁸	Germany; n = 2,051 started in 1995)*	Maternal stress and smoking, birth weight, breast-feeding, vaccinations, nutrition	Wheezing, atopic rhinitis, eczema
Avon Longitudinal Study of Parents and Children; <i>Children of the Children of the 90s</i> http://www.bristol.ac.uk/alspac/about/	United Kingdom (Bristol); n = 14,000 (started in 1991)*	Diet, sex, smoking, birth weight	Asthma, eczema, allergies
Isle of Wight birth cohort and 3rd Generation study ²⁰	United Kingdom (Isle of Wight), n = 1,456 (started 1989) Third generation, n = 406 (started 2010)	Sex, smoking, birth weight, BMI, method of feeding, vaccination, nutrition	Asthma, eczema, rhinitis, food allergy, lung function
GINIplus birth cohort (https://www.ncbi.nlm.nih.gov/pubmed/20082618)	Germany (Munich and Wesel), n = 5,991 (started in 1995)*	Parental and grandparental hypertension, diabetes, allergies	Atopic diseases
LISAplus birth cohort (https://www.ncbi.nlm.nih.gov/pubmed/12358337)	Germany (Munich, Leipzig, Bad Honnef, and Wesel), n = 3,097 (started in 1997)*	Parental and grandparental hypertension, diabetes, allergies	Atopic diseases

BMI, Body mass index.

*Third-generation cohort is planned.

(approximately 98%) DNA-M transmission is either dominated by the mother or transmitted evenly from the mother and father.²³

Challenges in recruitment of multigenerational cohorts

A major problem in long-term cohort studies is attrition of the cohort over time. Loss to follow-up is almost inevitable in studies that aim to recruit several generations sequentially. This can introduce bias as subsequent generations are self-selected and generally overrepresented by those with higher education achievements and socioeconomic status. The other important aspect is generalizability of findings (or lack thereof). The cohorts are usually based in one geographic location with an ethnic, economic, and exposure profile that might not be shared in other socioeconomic situations. Collaboration between cohorts with formation of new consortia or using existing birth cohort alliances provides an opportunity to address these challenges. A number of potential confounders that might need to be adjusted and some limitations of multigenerational trials when investigating epigenetic and environmental affects across generations are summarized in Table III.

WHAT MEDIATES CROSS-GENERATIONAL EFFECTS? (GENETICS, SHARED ENVIRONMENT, AND EPIGENETICS)

There are at least 3 possible mechanisms for the cross-generational transmission of disease risks. The first is that of shared familial environment. Environmental exposures, such as tobacco smoking, dietary patterns, and occupational, microbiome, and geographic exposures, such as air pollution and farm environments, are more likely to be shared among family members across generations and need to be accounted for in any studies. It is necessary to investigate multiple family members or whole families to differentiate intrafamilial homogeneity and heterogeneity from intergenerational homogeneity and heterogeneity. Typically, a birth cohort study includes only 1 (F1) generation child. In this case birth order itself or an interaction with the environment of a family provides some information on intrafamilial heterogeneity. However, it is necessary to recruit and study family members in successive generations with prospective ascertainment of exposures to capture this process.

Second, genetic inheritance through generations can explain familial resemblance in phenotypes. However, this cannot account for the increased risk of disease as a result of environmental

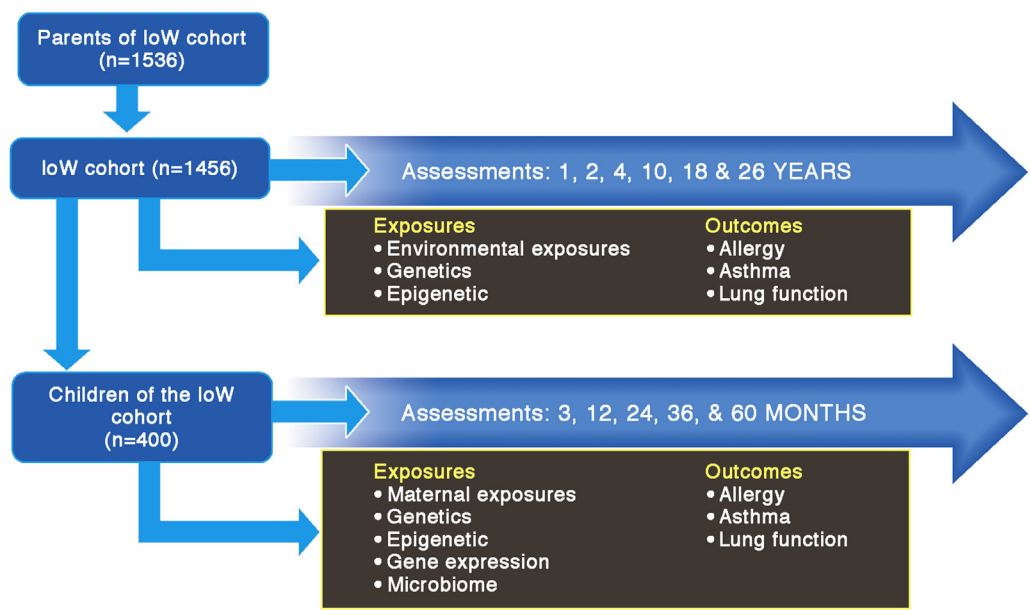


FIG 1. Three generations of the Isle of Wight birth cohort. The birth cohort participants were recruited in 1989-1990 and have been assessed at 1, 2, 4, 10, 18, and 27 years. Data and samples were collected from the parents of the cohort in 1989-1990. The third generation (children of the original birth cohort) have been recruited since 2010 and to date assessed at 3, 12, 24, 36, and 60 months.

TABLE III. Potential confounders and limitations of multigenerational studies

Generation	Confounders*	Opportunities	Limitations
First	Sex, maternal smoking during pregnancy, income, education, smoking, diet, BMI	Study genetic, environmental and gene-environment interactions for disease development	Assessment remains limited without parental genotype and phenotype information
Second	Sex, age, parental smoking, income, education, personal smoking, birth weight, gestational age, breast-feeding, family size, vaccinations, physical activity, diet, BMI	Parental influences (both genetic and <i>in utero</i> environmental) can be assessed.	Transgenerational effects (across multiple generation) cannot be assessed and inadequate for mechanistic understanding, especially epigenetic mechanisms.
Third	Sex, age, household smoking, personal smoking, income, education, birth weight, gestational age, breast-feeding, family size, vaccinations, physical activity, nutrition, diet, BMI	Grandparental (multigenerational) effects can be assessed in this generation.	To confidently evaluate epigenetic transfer of information, a fourth generation is required (see text).

BMI, Body mass index.
*With respect to outcomes, such as asthma, eczema, rhinitis, and lung function.

exposures of prior generations in the absence of continued exposure. The third possibility is that of epigenetic effects. Here a distinction needs to be made between intergenerational and transgenerational inheritance. As outlined above, in intergenerational inheritance the environment of the parent can directly affect germ cells of the offspring. A true transgenerational effect can only be defined if transmitted to the F2 (on the paternal line) or F3 (on the maternal line) generations and possibly future generations in the absence of further environmental exposure and with the exclusion of the possibility of germline mutations.^{24,25}

In experimental models a number of epigenetic mechanisms have been identified that have been shown to mediate epigenetic inheritance. For example, in yeast both prion-like proteins and transient expression of nonprion proteins have been shown to convey inheritance of traits independent of DNA.^{26,27} In

multicellular organisms, such as the nematode *Caenorhabditis elegans*, the RNA interference pathway results in non-DNA sequence-based heritable changes, and environmental exposures can result in heritable phenotypic changes.²⁸ In mammalian models, such as mice, a number of different types of nongenetic intergenerational or transgenerational inheritance have been observed.^{26,27} The most well-known examples of this are heritable nongenetic changes in expression of specific loci, such as at the Agouti viable yellow (*Avy*) locus affecting coat color and metabolic outcome caused by methylation silencing of an insertional mutation that results in ectopic *Agouti* expression,²⁹ which can be modified by maternal diet.³⁰

In another example Manikkam et al³¹ demonstrated transgenerational inheritance of adult-onset disease from exposure of gestating mice to an endocrine disruptor, the pesticide methoxychlor. The mechanism of this nongenetic form of inheritance

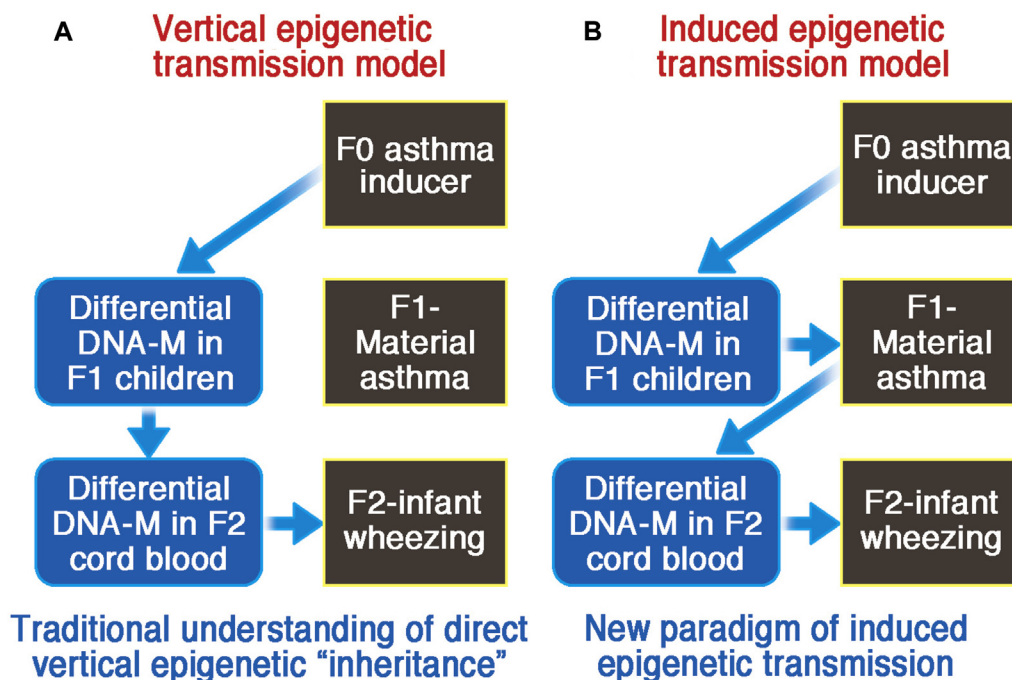


FIG 2. Vertical and induced epigenetic transmission models. **A**, The vertical epigenetic transmission model proposes direct transfer of epigenetic information from parent to child in successive generations. **B**, The alternative model, induced epigenetic transmission, proposes that maternal disease is an essential element in the chain of epigenetic transmission of information.

involved the transgenerational transmission of adult-onset disease susceptibility through epigenetic changes in the germline because epigenetic alterations in the sperm DNA of the F3 generation (great-grand offspring) could be observed after methoxychlor exposure of the F0 generation gestating female ancestors. This apparent transmission of epimutations has now been seen to be induced by a number of different environmental toxicants. However, an unknown factor is the transfer of persistent organic pollutants through the placenta from grandmothers (F0) to mothers (F1), as reported in multiple studies.³²⁻³⁴ In human subjects it has been demonstrated that between 23% and 43% of the concentration of persistent organic pollutants in mothers was explained by grandmaternal concentrations.³⁵ This leads to an exposure of F1 and thus a direct exposure of F3 oocytes when F1 mothers are pregnant with F2. Hence transfer of persistent toxins through the placenta means that for exposures, such as methoxychlor, intergenerational inheritance cannot be excluded because effects in the F3 generation might result from direct effects of the exposure.

As discussed by Miska and Furgeson-Smith,²⁴ transgenerational inheritance of epimutations such as observed by Manikkam et al,³¹ requires a substrate that transmits the information from one generation to the next and a mechanism for the transmitted information to be “read” or interpreted in the offspring to alter the phenotype. Evidence exists for a number of potential substrates, including incomplete germline DNA-M erasure during gametogenesis,³⁶ histone modifications,³⁷ or noncoding RNA species in gametes.⁸ Robust evidence of such mechanisms operating in human subjects is lacking. However, observations, such as microRNA expression in human spermatozoa from smokers compared with nonsmokers, suggests that similar mechanisms might operate.³⁸

CROSS-GENERATIONAL EFFECTS

In the F1 generation of the Isle of Wight cohort, we have shown that methylation of specific CpG sites is strongly associated with eczema, asthma, smoking, and obesity in F1 adults.³⁹⁻⁴⁵ In addition, maternal conditions during pregnancy, such as smoking, were associated with cord blood DNA-M, such as *AHRR* CpG cg05575921, which has become a well-established marker for past smoking.⁴⁶⁻⁴⁸

For epigenetic multigenerational inheritance,^{49,50} it is assumed that transmitted DNA-M escapes epigenetic reprogramming after fertilization, similar to an imprinted gene.⁵¹ However, this is not clearly established because it is reported that most DNA-M is erased, with only a few elements escaping erasure,³⁶ and as described above, other mechanisms for conveying information between generations can exist. For conceptual reasons, because the maternal lines F1 and F2 are already exposed in F0, proof of maternal intergenerational inheritance focuses on exposure in the F0 generation and epigenetic changes in the F3 generation. However, because there is no suggestion that an epigenetic leap occurs from F0 to F3, we would expect to find epigenetic associations between F1 and F2. In some previous studies these steps have not been investigated sequentially, and an association of exposure in F0 and phenotype outcome in F3 is taken as evidence of epigenetic transmission. In the Isle of Wight cohort study epigenetic associations between DNA-M in F1 and F2 were not commonly observed at a genome-wide scale (only approximately 1% of CpGs showed epigenetic associations after adjusting for multiple testing by controlling the false discovery rate of 0.05). In addition, for most CpG sites that showed associations with the respective parental CpG sites, the associations were eliminated once the offspring genotype was taken into consideration. Methylation quantitative loci (single nucleotide polymorphisms)

are a potential explanation of this phenomenon,⁵²⁻⁵⁴ indicating epigenetic associations were likely to be an epigenetic manifestation of genetic inheritance.

In contrast, animal studies addressing epigenetic inheritance either focused on F0-F1-F2 and not F3⁵⁵⁻⁵⁷ or did not investigate F1 and F2 after initial exposure of F0. These experiments often reported phenotypic changes in F1 and F2 but ignored processes in F1 and F2 as an explanation of reproduced DNA-M.^{31,58} On the basis of findings in our birth cohort, we believe it is critical to assess all generations from F0 to F3. It seems that it is the pathologic/immunologic condition arising from exposures/diseases in F0 that is likely to lead to an induced epigenetic transmission of a differential methylation of CpG sites from F1 to F3 in the maternal line. Hence we propose that the epigenetic dogma for transgenerational inheritance that "exposures in great-grandmothers during pregnancy may influence disease in their great-grandchildren, even in the absence of any exposure"⁵⁹ needs to be modified by the clause "but only in the presence of exposure-related diseases in F1 and F2" (Fig 2). Therefore we suggest that observations purported to be transgenerational might actually arise from transmitted disease. As Fig 2 shows, we assume that DNA-M can result in disease (asthma) or exposure (smoking) during pregnancy. Then exposure of the fetus to these diseases/exposures might initiate specific differential DNA-M in the offspring, which in turn can result in offspring experiencing diseases (eg, asthma) or behavior (eg, initiating smoking). We call this the induced epigenetic transmission model.

A limitation of this induced epigenetic transmission model is that it does not explain the paternal role or sex-specific inheritance, as seen in the Isle of Wight birth cohort from F0 to F1.²¹ Nevertheless, it is possible that the paternal role or sex-specific inheritance is related to methylation quantitative loci or genetic inheritance that results in differential methylation in offspring.

FUTURE DIRECTIONS

The question of the existence and importance of transgenerational inheritance in human subjects remains unresolved. The data in animal models are stronger for environmental exposure present in the F0 generation to have an effect in the F3 generation, supporting true transgenerational inheritance. However, the effect of disease in the mother has not been fully explored, which might explain in part these generational effects. Robust data in human subjects are lacking regarding the effect of environmental exposures in grandparents having an effect on disease development in grandchildren and whether this risk is mediated by epigenetic transfer of information across generations. The differentiation between intergenerational and transgenerational effects is important because there are many reasons (eg, direct transfer of environmental pollutants across the placenta) that need to be excluded before true transgenerational effects can be accepted. Further studies are required in human subjects, specifically multigenerational well-characterized cohorts with longitudinal information to avoid confounding by disease and treatment effects and recall bias. Family-based transgenerational studies will have the power to assess within-family factors, such as birth order and environmental exposures shared within the family, as well as between family factors. Larger studies with adequate sample size and/or collaboration between studies for replication and meta-analysis will provide much needed strength to these observations.

It is imperative to test the induced epigenetic transmission model in future studies, which proposes that maternal disease/exposure is critical for changes in methylation in the successive generation. If this model is correct, then the key to prevention of asthma and allergies in the next generation is to prevent asthma and allergies during pregnancy. Epigenetic effects might also depend on the sex of the child, and hence sex-specific analysis might reveal a differential effect. Family/sibling and sex-specific sibling effects can be studied by investigating several members of the family in multigenerational cohorts.

Lastly, future studies should also investigate the functional consequences of these epigenetic signatures transferred across generations to establish the clinical relevance of this phenomenon.

We acknowledge the help of all the staff at the David Hide Asthma and Allergy Research Centre in undertaking the assessments of 3 generations of the Isle of Wight birth cohort. We also acknowledge the participants and their families who have helped us over the last 2½ decades.

REFERENCES

- Arbes SJ Jr, Gergen PJ, Elliott L, Zeldin DC. Prevalences of positive skin test responses to 10 common allergens in the US population: results from the third National Health and Nutrition Examination Survey. *J Allergy Clin Immunol* 2005; 116:377-83.
- Manor O, Koupil I. Birth weight of infants and mortality in their parents and grandparents: the Uppsala Birth Cohort Study. *Int J Epidemiol* 2010;39:1264-76.
- McCarron P, Davey Smith G, Hattersley AT. Type 2 diabetes in grandparents and birth weight in offspring and grandchildren in the ALSPAC study. *J Epidemiol Commun Health* 2004;58:517-22.
- Smith GC, Wood AM, White IR, Pell JP, Hattie J. Birth weight and the risk of cardiovascular disease in the maternal grandparents. *Am J Epidemiol* 2010; 171:736-44.
- Kaati G, Bygren LO, Pembrey M, Sjöström M. Transgenerational response to nutrition, early life circumstances and longevity. *Eur J Hum Genet* 2007;15: 784-90.
- 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.
- Padmanabhan N, Jia D, Geary-Joo C, Wu X, Ferguson-Smith AC, Fung E, et al. Mutation in folate metabolism causes epigenetic instability and transgenerational effects on development. *Cell* 2013;155:81-93.
- 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.
- Chen Q, Yan M, Cao Z, Li X, Zhang Y, Shi J, et al. Sperm tsRNAs contribute to intergenerational inheritance of an acquired metabolic disorder. *Science* 2016; 351:397-400.
- Golding J, Northstone K, Gregory S, Miller LL, Pembrey M. The anthropometry of children and adolescents may be influenced by the prenatal smoking habits of their grandmothers: a longitudinal cohort study. *Am J Hum Biol* 2014;26:731-9.
- Northstone K, Golding J, Davey Smith G, Miller LL, Pembrey M. Prepubertal start of father's smoking and increased body fat in his sons: further characterisation of paternal transgenerational responses. *Eur J Hum Genet* 2014;22: 1382-6.
- Svanes C, Koplín J, Skulstad SM, Johannessen A, Bertelsen RJ, Benediktsdóttir 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* Aug 25, 2016 [Epub ahead of print]. PII: dyw151; PMID: 27565179.
- Balcázar AJ, Grineski SE, Collins TW. The Hispanic health paradox across generations: the relationship of child generational status and citizenship with health outcomes. *Public health* 2015;129:691-7.
- Toren K, Gislason T, Omenaas E, Jögi R, Forsberg B, Nyström L, et al. A prospective study of asthma incidence and its predictors: the RHINE study. *Eur Respir J* 2004;24:942-6.
- Burney PG, Luczynska C, Chinn S, Jarvis D. The European Community Respiratory Health Survey. *Eur Respir J* 1994;7:954-60.
- Clavel-Chapelon F. Cohort profile: the French E3N cohort study. *Int J Epidemiol* 2015;44:801-9.

17. Kelleher CC, Viljoen K, Khalil H, Somerville R, O'Brien J, Shrivastava A, et al. Longitudinal follow-up of the relationship between dietary intake and growth and development in the Lifeways cross-generation cohort study 2001–2013. *Proc Nutr Soc* 2014;73:118-31.
18. Weinmann T, Gerlich J, Heinrich S, Nowak D, Gerdes J, Schlichtiger J, et al. Establishing a birth cohort to investigate the course and aetiology of asthma and allergies across three generations—rationale, design, and methods of the ACROSSOLAR study. *BMC Public Health* 2015;15:1210.
19. Boyd A, Golding J, Macleod J, Lawlor DA, Fraser A, Henderson J, et al. Cohort profile: the 'children of the 90s'—the index offspring of the Avon Longitudinal Study of Parents and Children. *Int J Epidemiol* 2013;42:111-27.
20. Veerish VKHJ, Karmaus W, Zhang H, Mitchell F, Ewart S, Arshad SH. Third generation study: maternal and grand-maternal smoking in pregnancy and wheeze in children. *Allergy* 2014;69:326-453.
21. 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.e6.
22. Rehan VK, Liu J, Sakurai R, Torday JS. Perinatal nicotine-induced transgenerational asthma. *Am J Physiol Lung Cell Mol Physiol* 2013;305:L501-7.
23. 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.
24. Miska EA, Ferguson-Smith AC. Transgenerational inheritance: models and mechanisms of non-DNA sequence-based inheritance. *Science* 2016;354:59-63.
25. Heard E, Martienssen RA. Transgenerational epigenetic inheritance: myths and mechanisms. *Cell* 2014;157:95-109.
26. Halfmann R, Jarosz DF, Jones SK, Chang A, Lancaster AK, Lindquist S. Prions are a common mechanism for phenotypic inheritance in wild yeasts. *Nature* 2012;482:363-8.
27. Chakrabortee S, Byers JS, Jones S, Garcia DM, Bhullar B, Chang A, et al. Intrinsically disordered proteins drive emergence and inheritance of biological traits. *Cell* 2016;167:369-81.e12.
28. Rechavi O, Hourli-Ze'evi L, Anava S, Goh WS, Kerk SY, Hannon GJ, et al. Starvation-induced transgenerational inheritance of small RNAs in *C. elegans*. *Cell* 2014;158:277-87.
29. Morgan HD, Sutherland HGE, Martin DIK, Whitelaw E. Epigenetic inheritance at the agouti locus in the mouse. *Nat Genet* 1999;23:314-8.
30. Waterland RA, Travisano M, Tahiliani KG, Rached MT, Mirza S. Methyl donor supplementation prevents transgenerational amplification of obesity. *Int J Obes (Lond)* 2008;32:1373-9.
31. Manikkam M, Haque MM, Guerrero-Bosagna C, Nilsson EE, Skinner MK. Pesticide methoxychlor promotes the epigenetic transgenerational inheritance of adult-onset disease through the female germline. *PLoS One* 2014;9:e102091.
32. Vizcaino E, Grimalt JO, Fernandez-Somoano A, Tardon A. Transport of persistent organic pollutants across the human placenta. *Environ Int* 2014;65:107-15.
33. Li LX, Chen L, Meng XZ, Chen BH, Chen SQ, Zhao Y, et al. Exposure levels of environmental endocrine disruptors in mother-newborn pairs in China and their placental transfer characteristics. *PLoS One* 2013;8:e62526.
34. Porpora MG, Lucchini R, Abballe A, Ingelido AM, Valentini S, Fuggetta E, et al. Placental transfer of persistent organic pollutants: a preliminary study on mother-newborn pairs. *Int J Environ Res Public Health* 2013;10:699-711.
35. Hsu WW, Osuch JR, Todem D, Taffe B, O'Keefe M, Adera S, et al. DDE and PCB serum concentration in maternal blood and their adult female offspring. *Environ Res* 2014;132:384-90.
36. Hackett JA, Sengupta R, Zyllicz JJ, Murakami K, Lee C, Down TA, et al. Germline DNA demethylation dynamics and imprint erasure through 5-hydroxymethylcytosine. *Science* 2013;339:448-52.
37. Ost A, Lempradl A, Casas E, Weigert M, Tiko T, Deniz M, et al. Paternal diet defines offspring chromatin state and intergenerational obesity. *Cell* 2014;159:1352-64.
38. 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;7:432-9.
39. Patil VK, Holloway JW, Zhang H, Soto-Ramirez N, Ewart S, Arshad SH, et al. Interaction of prenatal maternal smoking, interleukin 13 genetic variants and DNA methylation influencing airflow and airway reactivity. *Clin Epigenet* 2013;5:22.
40. Ziyab AH, Karmaus W, Holloway JW, Zhang H, Ewart S, Arshad SH. DNA methylation of the filaggrin gene adds to the risk of eczema associated with loss-of-function variants. *J Eur Acad Dermatol Venerol* 2013;27:e420-3.
41. Everson TM, Lyons G, Soto-Ramirez N, Soto-Ramirez N, Holloway JW, Arshad H, 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.
42. Guthikonda K, Zhang H, Nolan VG, Soto-Ramirez N, Ziyab AH, Ewart S, et al. Oral contraceptives modify the effect of GATA3 polymorphisms on the risk of asthma at the age of 18 years via DNA methylation. *Clin Epigenet* 2014;6:17.
43. Zhang H, Tong X, Holloway JW, Rezwan FI, Lockett GA, Patil V, et al. The interplay of DNA methylation over time with Th2 pathway genetic variants on asthma risk and temporal asthma transition. *Clin Epigenet* 2014;6:8.
44. Soto-Ramirez N, Arshad SH, Holloway J, Zhang H, Schaubberger E, Ewart S, et al. The interaction of genetic variants and DNA methylation of the interleukin-4 receptor gene increase the risk of asthma at age 18 years. *Clin Epigenet* 2013;5:1.
45. Yousefi M, Karmaus W, Zhang H, Ewart S, Arshad H, Holloway JW. The methylation of the LEPR/LEPROT genotype at the promoter and body regions influence concentrations of leptin in girls and BMI at age 18 years if their mother smoked during pregnancy. *Int J Mol Epidemiol Genet* 2013;4:86-100.
46. Philibert RA, Sears RA, Powers LS, Nash E, Bair T, Gerke AK, et al. Coordinated DNA methylation and gene expression changes in smoker alveolar macrophages: specific effects on VEGF receptor 1 expression. *J Leukoc Biol* 2012;92:621-31.
47. Monick MM, Beach SR, Plume J, et al. Coordinated changes in AHRR methylation in lymphoblasts and pulmonary macrophages from smokers. *Am J Med Genet B Neuropsychiatr Genet* 2012;159B:141-51.
48. Shenker NS, Polidoro S, van Veldhoven K, Sears R, Gerrard M, Brody GH, et al. Epigenome-wide association study in the European Prospective Investigation into Cancer and Nutrition (EPIC-Turin) identifies novel genetic loci associated with smoking. *Hum Mol Genet* 2013;22:843-51.
49. Anway MD, Cupp AS, Uzumcu M, Skinner MK. Epigenetic transgenerational actions of endocrine disruptors and male fertility. *Science* 2005;308:1466-9.
50. Kanda J, Chiou LW, Szabolcs P, Sempowski GD, Rizzieri DA, Long GD, et al. Immune recovery in adult patients after myeloablative dual umbilical cord blood, matched sibling, and matched unrelated donor hematopoietic cell transplantation. *Biol Blood Marrow Transplant* 2012;18:1664-76.e1.
51. Osada T, Berglund P, Morse MA, Hubby B, Lewis W, Niedzwiecki D, et al. Co-delivery of antigen and IL-12 by Venezuelan equine encephalitis virus replicon particles enhances antigen-specific immune responses and antitumor effects. *Cancer Immunol Immunother* 2012;61:1941-51.
52. Bock C, Paulsen M, Tierling S, Mikeska T, Lengauer T, Walter J. CpG island methylation in human lymphocytes is highly correlated with DNA sequence, repeats, and predicted DNA structure. *PLoS Genet* 2006;2:e26.
53. 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.
54. Tycko B. Allele-specific DNA methylation: beyond imprinting. *Hum Mol Genet* 2010;19:R210-20.
55. Relton CL, Davey Smith G. Is epidemiology ready for epigenetics? *Int J Epidemiol* 2012;41:5-9.
56. Chinnery PF, Elliott HR, Hudson G, Samuels DC, Relton CL. Epigenetics, epidemiology and mitochondrial DNA diseases. *Int J Epidemiol* 2012;41:177-87.
57. 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.
58. Zhang H, Maity A, Arshad H, Holloway J, Karmaus W. Variable selection in semi-parametric models. *Stat Methods Med Res* 2016;25:1736-52.
59. Skinner MK. Endocrine disruptor induction of epigenetic transgenerational inheritance of disease. *Mol Cell Endocrinol* 2014;398:4-12.