

REVIEW ARTICLE

Does allergy begin *in utero*?Gabrielle A. Lockett¹, Johanna Huoman² & John W. Holloway^{1,3,4}

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Abstract

It has been recognized for centuries that allergic disease runs in families, implying a role for genetic factors in determining individual susceptibility. More recently, a range of evidence shows that many of these genetic factors, together with *in utero* environmental exposures, lead to the development of allergic disease through altered immune and organ development. Environmental exposures during pregnancy including diet, nutrient intake and toxin exposures can alter the epigenome and interact with inherited genetic and epigenetic risk factors to directly and indirectly influence organ development and immune programming. Understanding of these factors will be essential in identifying at-risk individuals and possible development of therapeutic interventions for the primary prevention of allergic disease. In this review, we summarize the evidence that suggests allergic disease begins *in utero*, together with possible mechanisms for the effect of environmental exposures during pregnancy on allergic disease risk, including epigenetics.

It is now well recognized that the prenatal environment plays an important role in disease risk in post-natal life: this is known as the developmental origins of health and disease (DOHAD) hypothesis (1). Evidence suggests that in early development the individual is not only more sensitive to environmental exposures, but also in a unique position to alter their developmental trajectory to match the predicted *ex utero* environment. Thus, exposures during *in utero* development can cause immediate reprogramming in response to temporary disturbances, leading to disease later in life. The role of developmental factors in allergic disease is now increasingly recognized. The correlation of disease-associated genetic variants with phenotypes measured in infancy and the effect of *in utero* environmental exposure on allergic disease risk, together with clear phenotypic differences at birth between those who do and do not go on to develop clinical manifestations of allergy, all suggest that allergic disease has its origins *in utero*.

Genes associated with allergic disease exert their effects during *in utero* development

A great number of studies have now defined genetic variants that affect an individual's risk of developing allergic disease (2). However, the point in organismal development at which these genetic variants start to influence allergic disease risk is

unknown. Genetic variants could alter disease risk through many potential mechanisms, for example modifying responses to post-natal environmental exposures, determining the level and type of inflammation that occurs in response to allergen exposure or determining the tissue response to such inflammation. However, it is becoming increasingly evident that many genetic variants associated with allergic disease exert their effect during early development. For example, the majority of SNPs identified in genome-wide association studies (GWASs) for lung function in adults also show the same direction of effect in 7–9 year-old children (3, 4), and of 23 SNPs with directionally consistent effects between children and adults, five were also associated with lung function in infants 5–14 weeks old (5); both results suggest that some lung function-associated genetic variants exert their effects *in utero*. *ADAM33*, a gene associated with asthma and bronchial hyper-responsiveness (BHR), is already expressed in the bronchi and surrounding mesenchyme of the 8–10 week-old embryo (6), and associated with lung function in infants (7), again suggesting this gene has a role in embryonic lung development, which may underlie its association with asthma.

Allergy-related differences already exist at birth

Foundations laid during the *in utero* period may have long-term effects on future health and disease, emphasizing the

importance of accurate regulation of early development, including immune maturation. Indeed, numerous studies show that there are marked phenotypic differences already apparent at the time of birth between individuals who do or do not go on to develop allergic conditions later in life.

For example, measurements of lung function have shown that children who go on to develop asthma have impaired lung function shortly after birth in comparison with healthy children (8). Slower growth of the foetus' abdominal circumference around mid-gestation is also associated with increased BHR in childhood (9). In the skin, reduced barrier function at both 2 days and 2 months of age – as measured by trans-epidermal water loss – has been shown to precede the development of eczema at 1 year of age, independently of *filaggrin* loss-of-function genetic variants (10).

Disparities in circulating factors and cells have also been demonstrated, as exemplified by elevated cord blood levels of long-chain polyunsaturated fatty acids (LCPUFAs), such as n-3 and n-6 PUFAs, which dose-dependently predict the development of respiratory allergies in children by 13 years of age (11). Likewise, epigenetic studies recently identified a cord blood CD4⁺ T cell DNA methylation signature at 96 CpGs that predicts the development of food allergy by 12 months of age (12).

Allergic diseases may originate from differences both in quantity and in quality of important immune cells and mediators, all of which may contribute to disease manifestations. Many such differences have been observed in cord blood cells at birth in those who subsequently go on to develop allergic disease compared to those who do not. For example, high cord blood serum IgE levels (13) and elevated numbers of IL-4⁺CD4⁺ T cells (Th2) (14) have been associated with increased risk of allergy development. Levels of membrane-bound CD14 on monocytes are lower in the cord blood of children with a higher risk of developing allergies later in life (15), and levels of soluble CD14 in amniotic fluid at mid-gestation are lower in children with subsequent eczema and sensitization at 12 months of age (16). TLR2 is more highly expressed in plasmacytoid dendritic cells at birth and one year of age (17), suggesting that the innate immune response may be hyper-responsive in those who subsequently develop allergy. Functional studies of the innate immune response in cord blood mononuclear cells of children with predisposition for allergies show that secretion of both inflammatory (IFN- γ , IL-6, TNF- α) and regulatory (IL-10) cytokines is increased at birth and diminished later in life upon Toll-like receptor activation (17, 18). These trajectories for allergic individuals in childhood point to downregulation of inflammatory responses over time, where an early hyper-responsiveness is modulated and ultimately results in a hyporesponsive state instead. Altogether, this suggests a role for variation in innate immune responses developing *in utero* and present at birth, which subsequently influences propensity to develop allergic disease.

With visibly dysfunctional innate immune responses, it intuitively follows that downstream adaptive immune responses may also be impaired at birth in individuals who

will go on to develop allergy. In children who will become allergic, proliferative responses of cord blood-derived T cells are indeed attenuated (19), and the aforementioned Th2 skewing has been established to lead to low Th1/Th2 and T_{reg}/Th2 ratios in cord blood (14). This skewing could be sustained by, for example, increased levels of the Th2-recruiting chemokines CCL17 and CCL22. In cord blood these chemokines predict development of allergic disease up to 6 years of age, and are specifically associated with allergic symptoms and sensitization, respectively (20). These findings are further supported by elevated expression of *CCL22* and the transcription factors *GATA3* (Th2) and *RORC* (Th17) in the cord blood of infants who later develop allergic disease (21).

Functional properties of the adaptive immune response also seem to be impaired in children who go on to develop allergic disease, as reflected by reduced allergen-induced cytokine responses in terms of IFN- γ (17, 19) and IL-5/IL-13 (19) levels in these children at birth. However, conflicting results have been reported regarding the same types of responses later in life, with significantly higher Th1- and Th2-associated cytokine responses at 12 months of age (19), in contrast to lower IFN- γ levels and higher IL-13 levels up to 5 years of age (17).

Altered proportions and functionality of effector T cells may contribute to the allergic response, but in the presence of an appropriate immunomodulatory response, this should theoretically be prevented. In the case of regulatory T cells, children with eczema do not have different T_{reg} counts at birth; however, their T_{reg} cells have reduced ability to suppress allergen-specific IL-4 responses (14). Other studies challenge these findings, both directly and indirectly displaying significantly lower numbers of CD4⁺CD25^{hi}FoxP3^{hi} T_{reg}s (22) and stable CD4⁺CD25⁺ T_{reg}s (23) in children who go on to be sensitized or develop other allergy-related symptoms in the first year of life. Placental expression of the T_{reg} master regulator transcription factor *FOXP3* is lower for children who subsequently become allergic (24), a finding corroborated by lower cord blood *FOXP3/CD3 γ* mRNA ratios in allergic children (13). Also, T_{reg} IL-10 secretion levels are lower in atopic individuals (22).

It is worth acknowledging that discrepancies between the above studies may originate from different study designs, different ethnic groups and environments, and different approaches to investigating various populations of immune cells. Regardless, these findings collectively point to impaired immune homeostasis, in terms of both numbers and function of immune cells, in allergy-prone individuals. Various aspects of the innate and adaptive immune responses as well as organ function may already be altered by birth, supporting the influence of early life on the development of atopy and allergic disease.

***In utero* exposures that influence allergy**

That differences are already evident at birth suggests that genetic and environmental factors during pregnancy and development have very early life influences on allergic disease

risk. A wide range of environmental exposures during pregnancy have been shown to influence the subsequent development of allergy in the offspring (Fig. 1).

While parental allergic disease is one of the strongest risk factors for allergy in the child, reflecting the effect of inherited genetic factors (as discussed above), it is clear that maternal allergy – and hence presumably an altered *in utero* environment – has an additional effect on the risk of allergy in the child. For example, the cord blood ratio of T_{reg} /Th2 cells is decreased in the children of allergic mothers (14). Maternal allergy also increases the risk of allergy in genetically identical mice: the offspring of peanut-allergic mothers are more likely to become allergic themselves (25), suggesting there is also a non-genetic component to parental allergy risk. Maternal atopy status also interacts with the *TLR1*, *TLR2* and *TLR10* genotype of the child to influence T_{reg} marker gene expression and Th2 cytokine levels in cord blood (26).

Besides allergic disease status, other maternal characteristics also influence the offspring's risk of allergy. Maternal obesity (body mass index of at least 35) and greater weight gain during pregnancy (at least 25 kg gained) both increase their child's risk of asthma, but not eczema or rhinitis, at age seven (27).

Maternal obesity also results in fewer eosinophils and $CD4^+$ T helper cells in the baby's cord blood, as well as altering cord blood cell responses to Toll-like receptor ligands and levels of cytokines (28).

Maternal smoking during pregnancy is one of the most well-studied *in utero* exposures and has been known for decades to increase the risk of multiple allergic diseases (29). Grandmaternal smoking during pregnancy with the mother has also been shown to increase the risk of asthma in her grandchildren (30), although the data are conflicting (31). Similarly, nicotine during pregnancy in rats impairs lung function in their offspring and grandoffspring (32). Interestingly, the increased risk of asthma with maternal smoking during pregnancy is attenuated by breastfeeding for at least 3 months in children who do not have recurrent lower respiratory tract infections (33), suggesting these risk factors for asthma may share common mechanisms. Maternal smoking during pregnancy also interacts with genetic variants in *IL13* to influence asthma risk (34), with genetic and epigenetic variation in *IL13* to influence airway reactivity (35), and with genetic variants at the asthma-associated 17q21 locus to influence early-onset asthma risk (36). A genomewide interaction study identified 27 SNPs

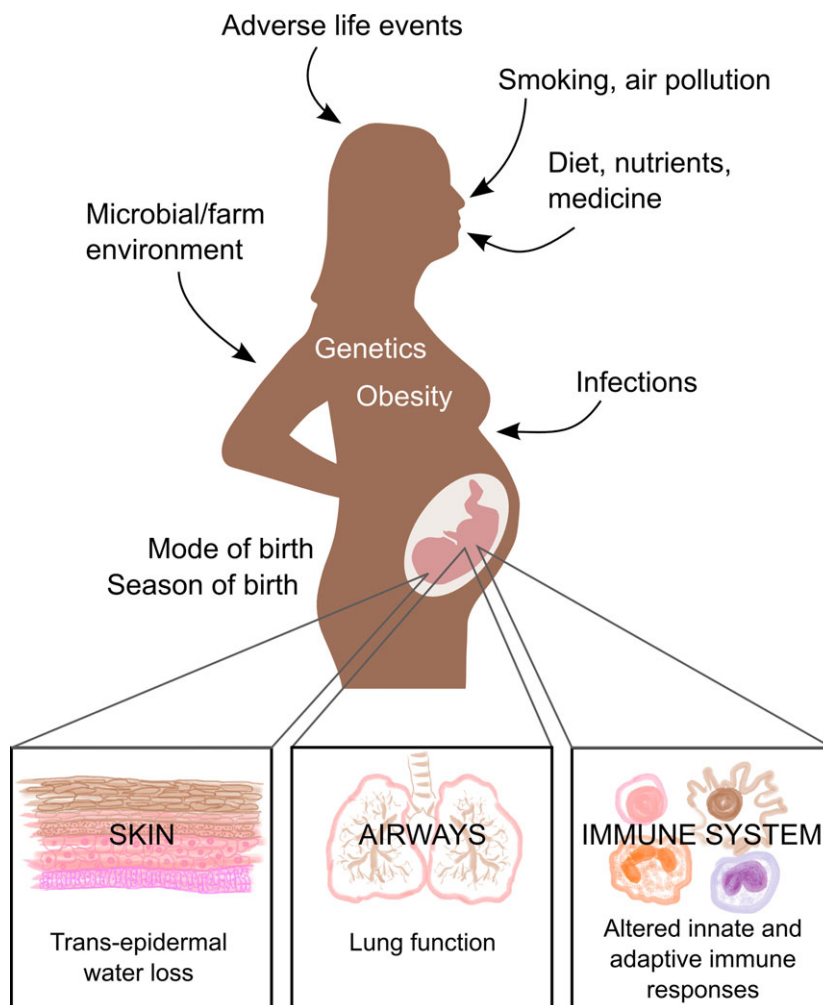


Figure 1 *In utero* environmental exposures influence subsequent risk of allergic disease. Exposures experienced by the mother during pregnancy, including a variety of intrinsic and extrinsic factors, result in differences in the child's subsequent risk of allergic disease. These trajectories are already apparent at birth and very early life in, for example, the rate of trans-epidermal water loss, lung function and immune system differences.

that interact with *in utero* smoke exposure to influence childhood-onset asthma (37).

During the later half of pregnancy, maternal experience of an adverse life event (separation/divorce, involuntary job loss, death of a close relative/friend) increases the child's risk of asthma and eczema up to 14 years of age, and this effect is stronger when the mother is not asthmatic (38). A mouse model study found that a single restraint stress on day 15 of pregnancy causes airway hyper-reactivity and allergic inflammation in their offspring, and pharmacological manipulation suggests the glucocorticoid pathway mediates this effect (39) – the same mechanism could mediate the effect of stress on allergy in humans.

Mode of birth also influences allergic disease risk: children born by Caesarean section have an increased risk of allergy; for instance, a recent study used mediation analysis to link mode of birth to the gut microbiome and eczema risk (40). Another study found that Caesarean-born babies have lower bacterial diversity and reduced *Bacteroidetes* in their intestinal microflora, as well as reduced Th1 responses, up to 2 years of age (41). Gestational age at birth influences the development of allergy too, as premature babies have an increased risk of wheeze and asthma during childhood, independent of birthweight (42).

The season in which a person is born influences their risk of allergic disease: those born in autumn and winter typically have higher risks of developing many allergic diseases including food allergy. This is also manifested at the immunological level, as exemplified by season of birth being the strongest predictor of cytokine production in cord blood cells (43). The primary stimuli underlying the season of birth effect on allergic disease are unknown, but may include ultraviolet light/vitamin D levels, pollen and viral infection timing, and seasonal variations in nutrition.

Maternal diet and micronutrients

The developing foetus relies on the mother not only to deliver sufficient molecular precursors for organismal development, but also to provide certain vitamins essential for immune system development. Maternal dietary restriction during pregnancy affects allergic disease risk, as demonstrated by individuals who were exposed to famine during early or mid-gestation having increased risk of obstructive airway disease (44). Maternal protein restriction also increases the risk of asthma: children with slower abdominal circumference growth at 11–19 weeks of gestation have greater BHR, and this is paralleled in rat models of protein restriction (9).

Fish and fish oil intake during pregnancy can be protective against allergy, although other studies find increased risk of allergy with higher fish intake. For example, one study found increased maternal fish intake protects against eczema at 1 year of age, and against atopy and atopic wheeze at 6 years, especially among children who were not breastfed (45). Maternal PUFA supplementation during pregnancy reduces atopic eczema and egg sensitization in the first year of life (46),

yet higher levels of LCPUFAs in cord serum are associated with increased risk of allergy (11). Another study showed that during the first trimester, maternal shellfish consumption increases the risks of wheezing and eczema, and fatty fish consumption increases the risk of asthma, although lean fish and total fish consumption did not affect allergic disease risk (47). An intervention study showed that when the mother eats more salmon during pregnancy, cord blood mononuclear cells display reduced Th2 (IL-4, IL-5 and IL-19) and Th1 (TNF- α and IL-2) cytokine levels upon stimulation – although this did not translate to an effect on the child's serum IgE levels, atopy or eczema risk at 6 months of age (48). Maternal fish oil supplementation decreases foetal *IL4* and increases *TGF- β* mRNA levels (49). Altogether, these studies suggest inconsistent effects of maternal fish and fish oil intake on allergic disease.

Another example of maternal intake of specific foods affecting offspring allergic disease is that daily consumption of natto, a traditional Japanese fermented soya bean food, is associated with reduced eczema risk at six months of age (50). The effect remained significant when controlling for intakes of other foods, suggesting that natto consumption is not a surrogate marker of a protective maternal dietary pattern. The authors hypothesize that the protective effects of *Bacillus subtilis* – the bacterium used to ferment natto – on intestinal health and perhaps the mother's immune system, underlie the protective effect of maternal daily natto consumption against the child's risk of eczema.

Folate is another major dietary nutrient required for early development, which provides the methyl precursor groups for DNA methylation and is recommended to pregnant women for its role in preventing congenital anomalies. While the majority of studies report that increased folate levels are associated with increased risk of allergic disease, there are a minority of studies reporting findings in the opposite direction. Maternal and plasma folate levels (51) are positively associated with the risk of asthma at 3 years of age, and this effect is paralleled in mice fed methyl donor-rich diets during gestation (52). However, other studies have found that higher folate levels are associated with decreased risks of atopy and wheeze, and serum IgE levels (53), or have no effect either way on allergic disease risk after one year of age (54). One study reports that while higher folate supplementation increases the risk of eczema, interestingly there is a nonlinear association between cord blood folate levels and risk of allergic sensitization at one year of age, with both extremes increasing sensitization risk (55). Mendelian randomization studies have variously reported that the *MTHFR* functional genetic variant C677T, which impairs a key enzyme in folate metabolism, has no effect on the risks of allergy, atopy or asthma (56), or increases the risk of atopy (57). While maternal folate supplementation is of great importance in preventing congenital anomalies such as spina bifida, these reports together suggest that any increased risk of allergic disease with folate supplementation may be transient and confined to the early years.

Maternal plasma selenium levels during early pregnancy are positively associated with the risk of wheeze up to age 2 years;

however, maternal selenium levels no longer have an effect on wheeze at 5 years of age (58). The Mendelian randomization approach, in this case utilizing the child's genotype at a SNP within *GPX4* as a proxy for selenium levels, revealed that higher maternal blood selenium levels only reduce the risk of asthma and wheezing at age seven in genetically at-risk children homozygous for the minor allele at this SNP (59).

Levels of other vitamins during pregnancy, from both diet and supplements, also affect allergic disease risk. Total maternal intake of vitamin K is positively associated with ever having asthma and having asthma at 7 years of age, and maternal intakes of vitamins A and E are weakly inversely associated with the risk of childhood allergic rhinitis (60). Higher maternal dietary vitamin C levels reduces the risk of any allergic disease and wheeze, and higher maternal copper consumption reduces the risk of eczema, wheeze and any allergic disease (61). Maternal intakes of vitamins D and E during pregnancy alter neonatal airway epithelial cell responsiveness (62) and are inversely associated with the risk of having doctor-diagnosed asthma at age 10 (63). The mother can also obtain vitamin D through sunlight exposure, constituting a possible mechanism for the effect of season of birth on allergic disease risk. Additionally, the gut microbiome has been suggested to be essential for the metabolism and production of some vitamins (64), suggesting that maternal effectual levels of some vitamins may be determined by a combination of dietary intake and microbiome composition.

Maternal medicines

Maternal use of paracetamol (acetaminophen) in late pregnancy has been associated with increased risk of asthma, wheezing and higher total serum IgE in the offspring (65). Interestingly, the effects of maternal paracetamol during pregnancy on offspring allergy risk are modified by maternal genotype at antioxidant-encoding genes (66). However, whether the association of maternal paracetamol intake and childhood asthma represents a causal relationship remains to be firmly established. A recent study of perinatal exposure to paracetamol in mice failed to provide evidence to support a causal relationship (67). Other medicines besides paracetamol have been shown to influence allergic disease. An analysis of over half a million Swedes found evidence that maternal use of gastric suppression medicine during pregnancy increases the child's risk of childhood asthma (68). However, as with paracetamol use, it is difficult to exclude reverse causation.

The microbial and chemical environment

The microbial environmental exposure most widely documented as influencing allergic disease is exposure to a farm environment, both prenatally and post-natally. Numerous studies have documented the reduced rates of allergic disease in individuals and cultures with greater exposure to farming and farm animals (69).

Exposures to specific micro-organisms and parasites on allergic disease have also been shown to have protective effects.

For example, in mice, mating during the Th1 or regulatory phase of maternal helminth infection, but not the intervening Th2 phase, protects the offspring against allergic airway inflammation (70). Maternally derived IFN- γ during the acute phase of infection, rather than any transfer of helminth antigens, is proposed to underlie this protective effect. When mice are exposed to the bacterial membrane chemical lipopolysaccharide (LPS) before conception or during pregnancy, their pups have higher levels of IFN- γ and suppressed IgG and IgE responses to sensitization, yet airway hyper-responsiveness is unaltered (71). These mouse models suggest that maternal exposure to bacteria and helminths during pregnancy produces maternal immune responses that are protective against allergy in their offspring, in concordance with the farm effect. Conversely, maternal urinary tract infection during pregnancy increases the child's risk of asthma before age six, although this is perhaps due to the antibiotics used to treat urinary tract infections (72).

In an intriguing parallel to the farm effect, a comparison between Papua New Guinean and Australian cord bloods found that antigen-presenting cells from Australian cord bloods were more reactive and less abundant (73). The difference was attributed to the general environment experienced by the mothers during pregnancy: Papua New Guinea is more rural and scores much lower than Australia on indices of human development.

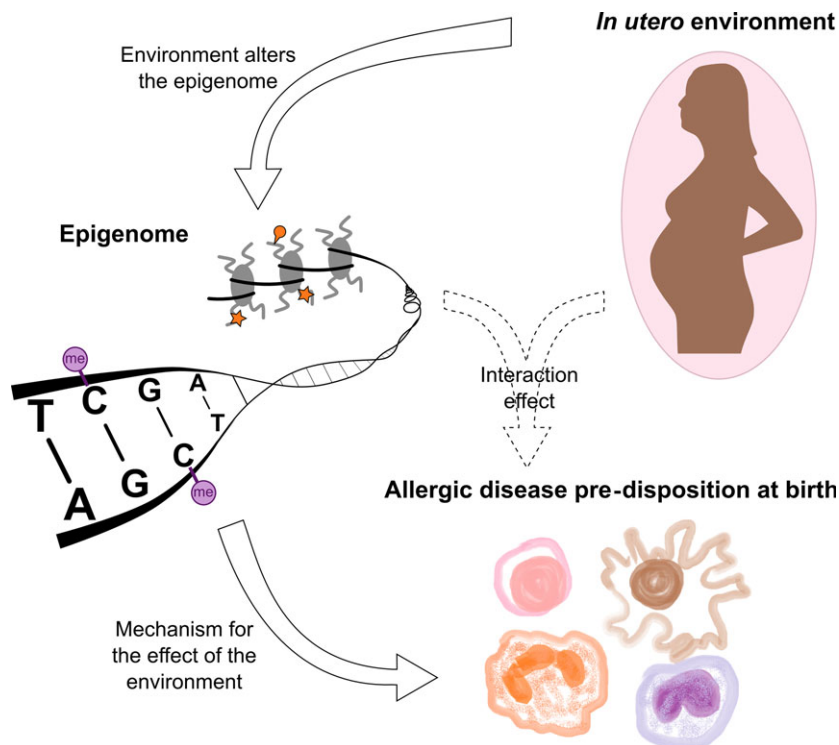
Maternal exposure to pollutant chemicals has been hypothesized to influence the child's allergy risk. There are many examples of current exposure affecting allergy, but prenatal exposure too can influence allergic disease. Higher levels of exposure to nitrogen dioxide, soot and particulate matter of diameter $\leq 2.5 \mu\text{m}$ (PM_{2.5}) at the child's birth address were associated with increased risk of asthma at age 8 (74). Increased maternal exposure to PM₁₀ during the last 3 months of pregnancy increased IL-1 β levels in cord blood, and during the last 3 days of pregnancy, reduced levels of IL-10 (75). *In utero* exposure to bisphenol A (BPA) and phthalates increases the child's risk of asthma and respiratory tract infections during childhood (76, 77).

Mechanisms for *in utero* effects

Developmental programming is the concept that at key sensitive windows during development, the individual is especially susceptible to being nudged onto a slightly different developmental trajectory, which then affects their long-term risk of allergic disease, in accordance with the DOHAD hypothesis. Prenatal exposures can therefore have disproportionately large effects on allergic outcomes, such as the effects of maternal protein restriction on lung development (9).

While some grandparental exposures can influence allergic disease, such as grandmaternal smoking (78), these do not necessarily suggest transgenerational epigenetic inheritance has a role in allergic risk. Inside the pregnant F₀ woman, the developing foetus of her F₁ daughter already contains the oocytes that will become the F₂ grandchildren; therefore, the

Figure 2 The *in utero* environment can both alter and interact with the epigenome, to cause differences in allergic disease predisposition at birth. Environmental factors, such as those described in Figure 1, can alter the epigenome, suggesting epigenetics as a mechanism for the effects of the *in utero* environment on allergic disease predisposition. The *in utero* environment can also interact with the epigenome to influence allergic disease risk.



first genuinely non-exposed generation would be the F₃ great-grandchildren. To our knowledge, effects of *in utero* exposures on allergic disease in the great-grandchildren have yet to be demonstrated. An analysis of intergenerational effects of maternal smoking on child DNA methylation concluded that even to the next generation, epigenetic differences are a consequence of sustained *in utero* exposure rather than inherited epigenetic marks (79).

Epigenetics, the set of processes that regulate gene expression without altering the DNA sequence, are strong candidates for the biochemical mechanism mediating *in utero* effects on allergic outcomes. The most frequently studied epigenetic processes are DNA methylation and histone post-translational modifications, although microRNAs and many other processes are also part of this category. Aside from the role of epigenetics in organismal development, the epigenome also responds to environmental stimuli and alters gene expression, sometimes with effects in some cases lasting decades (80, 81). In fact, many of the environmental stimuli discussed above to influence the risk of allergic disease are also associated with epigenetic differences.

A number of examples, and there are no doubt many more still to be uncovered, show that epigenetic mechanisms could well mediate the effect of a wide range of *in utero* exposures on allergic disease risk. Epigenetic processes could provide the mechanism for the effects of the *in utero* environment on gene expression and in addition can interact with maternal environmental exposures to influence allergic outcomes (Fig. 2). For example, Patil and colleagues showed that maternal smoking interacts with genetic and epigenetic variants of *IL13* to influence airflow limitation and airway responsiveness at age 18 (35).

Epigenetic examples

Maternal allergy likely has a strong genetic component, yet even in an inbred mouse line, maternal allergy alters DNA methylation of the *IL4* promoter in the pups (25). A mechanistic study found that sex, parental atopy or allergy and maternal smoking are all associated with reduced numbers of cord blood T_{reg} cells (with demethylation of *FOXP3* in the T_{reg}-specific demethylated region), which are in turn associated with higher risks of developing eczema and food allergen sensitization by 1 year of age (23).

Smoking during pregnancy has defined and reproducible effects on the epigenome: it affects DNA methylation at specific CpGs in the offspring at birth (82) and at 17 years of age (83), including at allergy-associated genes such as *TSLP* (84). Maternal prenatal smoking also alters the microRNA profile of the placenta (85). In rats, maternal nicotine during pregnancy alters histone H3 and H4 modifications in the foetal lung and gonad (32).

Maternal adverse life events, which act via the glucocorticoid pathway to influence allergy risk (39), may well induce epigenetic alterations to genes in this pathway, as DNA methylation within the *glucocorticoid receptor* gene is responsive to social stimuli (86).

Famine experienced by the mother during pregnancy has persistent effects on DNA methylation that occur primarily within regulatory regions, within moderately methylated regions and within genes expressed during early development (87). Methyl donor supplementation in mice is also associated

with differential methylation, including at *Runx3*, a regulator of airway disease (52).

Air pollution has well-recognized effects on the epigenome (reviewed by 88). In cord blood cells, there are over 30 sequences differentially methylated according to maternal polycyclic aromatic hydrocarbon exposure levels, including a CpG island in *ACSL3* whose methylation is also associated with asthma before age 5 (89). Prenatal exposure to the pollutant phthalate may increase the risk of childhood asthma via a mechanism of altered DNA methylation (90).

Conclusions

There is strong evidence that the origins of allergic disease risk lie in *in utero* development: allergy-associated genetic variants, along with the uterine environment – influenced by maternal

exposures and experiences – produce phenotypic differences already visible at birth between those who go on to develop allergic disease and those who do not. Our current best predictors of childhood asthma retain a degree of inaccuracy, suggesting we have not yet captured the full range of variations, exposures and interaction effects that explain allergy risk. Yet despite this, the greater ability to set disease trajectories in early development points to interventions very early in life, perhaps even *in utero*, as offering the greatest opportunity for primary prevention of allergic disease.

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