



Role of epigenetics and DNA-damage in asthma

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Purpose of review

Although asthma is a common disease worldwide, its pathogenesis remains to be fully elucidated. There is increasing evidence of the interaction between epigenetics, DNA-damage, and environmental allergens in the development of asthma. In this review, we will focus on the role of epigenetics and DNA-damage in asthma.

Recent findings

There is growing evidence of environmental allergens, particularly house dust mite, stimulating oxidative DNA damage in airway epithelial cells. The repair of this DNA damage has been implicated in the secretion of Th2 cytokines and the induction of allergic inflammation.

Summary

Studies of the role of epigenetics, DNA-damage, and environmental allergens have begun to reveal the their complex interactions and their roles in the development of asthma. Further study in these areas may lead to novel prevention and treatment approaches.

Keywords

asthma, DNA-damage, epigenetics

INTRODUCTION

Asthma is a common disease worldwide that affects about 7.5% of the adult population and is associated with high morbidity in the setting of inadequate or poor disease control [1^{••}]. It is a chronic inflammatory disorder that is characterized by airway symptoms, reversible airflow obstruction, and airway hyper-responsiveness [2[•],3]. Although most of asthma begins in childhood, its pathogenesis remains to be fully elucidated [3].

Asthma is likely transmitted by multiple genes. Further complicating our understanding is the interaction between genetic factors and environmental influences. One of the major reasons epigenetics has become a topic of great interest is that genome-wide association studies have been unable to explain more than a fraction of the phenotypic variance in asthma [4]. The notion that genetics alone do not account for the cause of asthma has led to the exploration of alternative sources of phenotypic variance [5].

In addition to genetics, environmental factors have also been implicated. Asthma has also been associated with oxidative stress. Immune cells in asthmatic patients generate a variety of genotoxic reactive oxygen and nitrogen species (RONS) [6,7], all of which contribute to DNA damage. In this review we will focus the role of epigenetics and DNA-damage in asthma.

THE EPIDEMIOLOGY OF ASTHMA

There are many factors that play a role in the cause of asthma, including inheritance, the general environment, and T_H2 immunity. However, there has been no underlying mechanism tying these causes together [1^{••}]. Asthma concordance in monozygotic twins has been found to be approximately 50% [8], suggesting that nongenetic factors play a significant role [9]. There is evidence of complex gene–environment interactions as seen in the increased risk of asthma in offspring of mothers who smoked during pregnancy [10]. This is in contrast to individuals who were exposed to rural environments in utero, which seemed to have a protective effect against the development of asthma [11,12]. In addition, there are sex-specific differences as there is higher a prevalence of asthma in prepubescent boys and the highest prevalence among girls after puberty [13]. Finally, imprinted genes may play a role as there are

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KEY POINTS

- Epigenetics may partially explain the variety of phenomena seen in asthma, including phenotypic discordance, sex effects, and transgenerational effects of environmental exposures.
- Studies have found that house dust mite-induced asthma causes oxidative damage and DNA double-strand breaks in lung epithelium.
- Further work is needed in evaluating the roles of epigenetics, DNA-damage, and environmental allergens in the development of asthma.

parent-of-origin effects in the susceptibility to asthma and elevated total serum IgE [14–18].

Association studies have been used to determine the relationship between specific disease characteristics and the presence of certain alleles of a particular DNA marker [19]. Association studies have identified approximately 50 replicated genes, several by using linkage and fine mapping, and one gene by genome wide association studies. Less than 10% of the heritability of asthma is predicted by these gene variants [20]. One limitation of these studies is that they have primarily studied white populations and to a lesser degree minority populations (Hispanics and African-based) despite these groups suffering disproportionately from both asthma morbidity and mortality [21–24].

INTRODUCTION TO EPIGENETICS

Epigenetics refer to a heritable phenotypic change that is not because of changes in the genome, meaning that there are heritable changes in gene expression in the setting of noncoding changes in DNA. Hence, epigenetic mechanisms affect the expression of genes without even changing the nucleotide sequence [1[¶]].

These mechanisms usually involve posttranslational modifications of histones, DNA methylation, and the expression of noncoding RNAs [3,25]. After replication, methylation, a covalent modification of DNA, mostly affects position five of the pyrimidine ring of cytosines near the dinucleotide sequence CpG [26]. CpG islands, clusters enriched in CpG, are typically found in locus control and promoter regions.

Methylation is a key process in altering gene expression. Hypermethylation of cytosines within CpG islands in gene promoters leads to gene silencing, whereas hypomethylation leads to active transcription [27–31]. There are many possible trigger mechanisms for methylation, including:

recognition of specific target DNA sequences, RNA interference, and posttranslational modifications of chromatin structures [32,33]. Studies have revealed that not only are CpG islands but regions attached to CpG islands, CpG shores, are functionally important in methylation control [34].

A recent study evaluated the peripheral blood mononuclear cells in allergic rhinitis individuals before and after grass pollen exposure in an environmental exposure unit. They found that this small cohort of patients underwent rapid and significant DNA methylation changes (at least 2%) in response to allergen exposure, which did not occur in nonallergic controls. Baseline SLFN12 expression predicted symptom severity. This suggests that epigenetic changes in the blood could be a proxy for similar changes in target tissues [35[¶]].

Poole *et al.* [36] have demonstrated that that gene expression in nasal epithelia can be a proxy measure for gene expression of the lower airway epithelium. Yang *et al.* [37^{¶¶}] have shown that more than one third of methylation changes can be generalizable to atopic asthma regardless of other characteristics including age, sex, race/ethnicity, and specific exposures.

A genome-by-environmental study evaluating a SNP (rs117902240) and dust mite exposure on the FEV1 in children with asthma found a significant interaction effect on FEV1 with dust mite allergen level. This SNP is located on chromosome 8q24, which is adjacent to a binding site for CCAAT/enhancer-binding protein β , which is a transcription factor that forms part of the Interleukin (IL)-17 signaling pathway. The SNP was positively associated with FEV1 in children exposed to low dust mite allergen levels, but negatively associated with FEV1 in children exposed to high levels [38[¶]].

Promoter regions are also important in gene expression. These regions are characterized by the presence of various histone modifications including methylation and acetylation (Fig. 1), which in turn promote certain gene expression [39,40].

EPIGENETICS, DNA DAMAGE, AND ASTHMA

Epigenetics may help explain a variety of phenomena in asthma, including phenotypic discordance, difference in age of onset of symptoms, sex effects, parent-of-origin effects, sporadic occurrence of disease, and transgenerational effects of environmental exposures [34].

Allergic asthma, the most common form of asthma, can be triggered by allergens. Associated allergens include house dust mite, animal dander, plant pollen, and fungal spores [41]. These aeroallergens

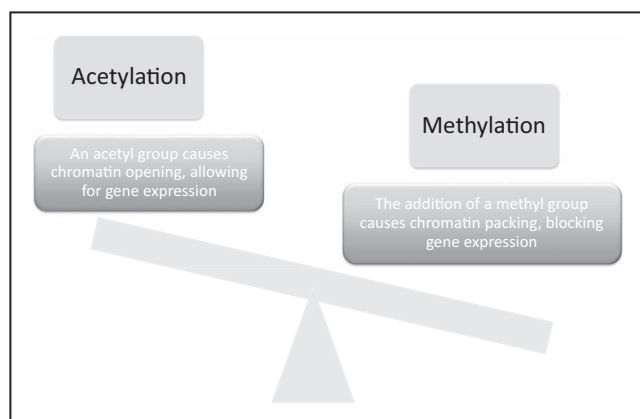


FIGURE 1. Two main epigenetic modifications are implicated in regulating gene expression. DNA is packaged around proteins known as histones which allow for the packaging and regulation of expression of genes. The DNA and histone combined structure together is known as chromatin. The attachment of an acetyl group allows for the unraveling of chromatin, exposing DNA for expression. Conversely, the addition of a methyl group causes the chromatin packing and therefore blocking access to gene expression. Genes may be hyper-methylated or hypo-methylated further allowing for the furling or unfurling, respectively, of DNA.

can negatively impact the bronchial epithelium and contribute to the development of asthma.

Studies have now shown that environmental exposures to certain allergens can, in fact, influence genetic expression (Fig. 2). An experimental study investigating heritability of epigenetic mechanisms and epigenetic effects on lung function after prenatal nicotine exposure in rats suggested that epigenetic patterns are possibly inherited [42]. Although the effect of allergic sensitization on airway cells to aeroallergens has had significant investigation, there have been few studies elucidating the negative impact of allergens on lung airway epithelial cells [43²²].

To further elucidate the effect of prenatal and early postnatal exposures to environmental factors and their effect on the development of allergic disease, Jahreis *et al.* have shown that maternal phthalate (a common plasticizer) exposure increases the risk for allergic airway inflammation in offspring. Using a murine transgenerational asthma model, they propose that the disease-promoting effect was mediated by a phthalate metabolite-induced global DNA hypermethylation in CD4⁺ T cells in the offspring. This finding was supported by the observation that treatment with a DNA-demethylating agent decreased allergic airway inflammation. Among the 13 transcriptionally downregulated genes linked to promoter or enhancer hypermethylation, the

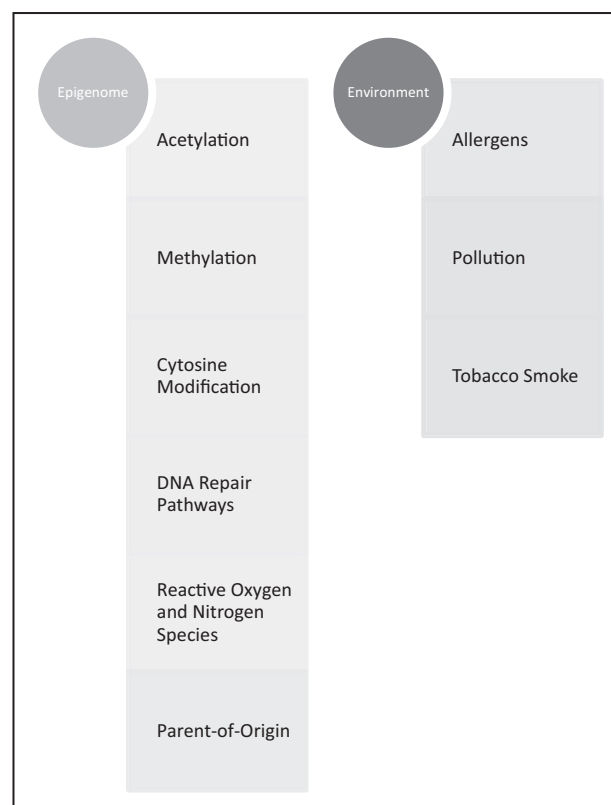


FIGURE 2. Epigenomic and environmental factors that affect gene expression.

GATA-3 repressor zinc finger protein 1 (Zfp1) was the likely mediator of the enhanced susceptibility for T_H2-driven allergic asthma [44²¹].

As the mechanism of asthma inception is not well defined, DeVries *et al.* evaluated DNA methylation in cord blood mononuclear cells from a small cohort of patients and found that SMAD3 promoter hypermethylation was selectively increased in asthmatic children of asthmatic mothers. Further this alteration in the methylome also was associated with increased LPS-induced IL-1 β . These findings were associated with an increased childhood asthma risk [45²¹].

In addition, allergen exposure can also lead to RONS, such as hydroxyl radicals, superoxide, peroxides, peroxyne, and nitric oxide [6,7]. RONS can lead to damage of a variety of biologic products including proteins, lipids, and nucleic acids [46], all of which can contribute to increased asthma severity [6]. It has been demonstrated that pollen extracts induce the creation of intracellular reactive oxygen species in epithelial cells [47].

A recent study found that house dust mite challenge to lung tissue led to increased lung levels of oxidative damage to proteins, lipids, and nucleic acids. House dust mite induced DNA double-strand breaks (DSBs) were revealed by increased levels of

the DSB marker gamma histone 2AX foci in bronchial epithelium. These studies also revealed increased levels of DNA repair proteins and apoptosis as evidenced by caspase-3 cleavage, catalase-activated DNase levels, and terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling staining [48²²].

Once DNA is damaged, there are two main DNA DSB repair pathways: homologous recombination (HR) and nonhomologous end-joining (NHEJ) [48²²]. HR uses a homologous DNA strand as a template to replace damaged genetic information [49]. Nonhomologous end joining, however, requires DNA-dependent protein kinase catalytic subunit for the detection and direct joining of DNA ends [50]. DNA damage repair requires several players including sensors, transducers, and effectors to effect DNA repair, cell-cycle arrest, and cell-death [51].

Defective DSB repair plays a significant role in immune dysfunction, cancer, and asthma [52]. An early and crucial step in the response to DNA DSBs is the phosphorylation of H2AX to gammaH2AX near the breakpoint. This allows for the recruitment of downstream damage response and DNA repair factors [53]. Rad51 and Ku70 are DNA repair proteins essential in the HR and NHEJ repair pathways, respectively [54,55]. Studies have found that cells deficient in either of these repair proteins have an increased sensitivity to DNA damage-induced cytotoxicity [56,57].

Studies have previously reported that exposure of allergic mice to ragweed pollen extract induces accumulation of 8-oxo-7,8-dihydroguanine (8-oxoG) damaged DNA base in the genomic DNA of airway epithelial cells (AECs) [58]. 8-oxoG is one of the most common oxidation products and is preferentially repaired by the DNA base excision repair pathway [59]. As guanine has the lowest redox potential among nucleic acid bases, the primary target of ROS is guanine [60]. The DNA repair enzyme OGG1 excises 8-oxo-G from the genome, and the free base complexes with OGG1, initiating OGG1/Ras/GTP-mediated activation of nuclear factor kappa-light-chain-enhancer of activated B cells, which drives the expression of multiple mRNAs, and allergic airway inflammation [58,61,62].

Ragweed pollen extract challenge in Ogg1-deficient (Ogg1D) mice has been shown to induce significantly less recruitment of eosinophils and induction of allergic airway inflammation. A recent study evaluated DNA damage in response to house dust mite in mice. They found that house dust mite-induced asthma causes oxidative damage and DNA DSBs in the lung. However, there have been no studies evaluating the degree of DNA or RNA

damage in patients with allergic and nonallergic rhinitis [48²²].

Chan *et al.* have demonstrated that direct exposure of human bronchial epithelial cells to house dust mite leads to ROS production and increased DNA damage in cells. In fact, a defect in DNA DSB repair worsened DNA damage, proinflammatory cytokine production, and apoptosis in the bronchial epithelium [48²²].

Generation of glutathione disulfide (oxidized glutathione [GSSG]) is another marker of oxidative stress. A recent study has found that a variety of allergenic extracts stimulate TLR-4 dependent and independent ROS and GSSG generation. Ragweed pollen extract (RWPE) can also induce TLR4-dependent oxidative DNA damage as evidenced by increased comet tail moments, which is a measure of oxidative DNA damage. In allergic rhinitis human patients sensitized to RWPE undergoing RWPE challenge, these patients had increase intranasal levels of 8-hydroxydeoxyguanosine at 30 min compared to saline challenge [63].

A recent study proposes that a state of sustained oxidative stress in the airways because of repeated recruitment of activated ROS-generating neutrophils in the setting of pollen exposure is a possible way that the immune response is shifted towards an allergic phenotype [64²³].

LIMITATIONS AND FUTURE DIRECTIONS

With advancing technologies, especially in the science of genomics, the possibility of additional data regarding the association of gene expression and asthma is likely. However, at this time, the technology does have limitations. One major weakness of the initial genome wide association studies is that these studies relied on platforms that provide limited genomic coverage. Furthermore, these studies were mostly designed to address cancer-related differential DNA methylation. DNA methylation in nasal epithelia could be used as a biomarker of exposure or disease after validation in larger cohorts [37²⁴]. Further, Barton *et al.* have shown that higher methylation of GATA3 CpGs at birth was associated with reduced risk of asthma in childhood. This could serve as a possible biomarker that could be used in the diagnosis and treatment of infants at risk of developing atopic disease [65²⁵].

Future studies may prove transformational to the field if they incorporate well phenotyped, longitudinal mother-child cohorts with an in-depth assessment of environmental exposures in patients in early infancy. This would allow for a comparison of fetal methylome and the methylome and exposures of the mother [39]. Further oxidative damage

studies could potentially evaluate challenges with noncross-reactive allergenic extracts. As it has been proposed that allergenic extract-induced ROS generation and oxidative DNA damage 'is not limited to a stochastic oxidative insult induced by allergen-epithelial interaction but a process that is initiated by innate immune receptors', further work exploring antagonists of TLRs and their adaptors in suppressing allergen-induced oxidative stress and DNA damage is needed [63]. In addition, further research in the molecular mechanisms of base excision repair signaling may provide the means of reversing DNA damage.

CONCLUSION

Asthma remains a common and heterogeneous disease with significant associated healthcare costs. The present review highlights the potential role of epigenetics, DNA damage, and environmental allergens in the development of asthma. A new emerging concept is that allergens stimulate oxidative stress and oxidative DNA damage in epithelial cells, and repair of oxidatively damaged DNA by DNA glycosylases modulates cellular signaling in epithelial cells that stimulates secretion of Th2 cytokines and induces allergic inflammation. As our understanding of the relationship of the genome and the epigenome evolves, we come closer to developing new and better therapeutic modalities to treat and possibly prevent this disease.

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Conflicts of interest

There are no conflicts of interest.

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