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Abstract	Numerous genetic, environmental, and hormonal factors underlie the pathogenesis of asthma leading to an abnormal antigen–antibody reaction inducing a vago-vagal axon reflex-mediated bronchoconstriction.Asthma has a complex and largely undefined genetic background. Similar to most complex diseases, its heterogeneous phenotype is thought to result from the interaction between multiple genes and environmental factors.It has been estimated that 73% of asthma determinants are genetic. Moreover, genetic variation is also thought to account for approximately 60–80% of the inter-individual variability in therapeutic response to medical treatments.Epigenetics seems to explain the corticosteroid resistance in patients with COPD.Over 25 genes have been hypothesized to be involved in asthma pathogenesis. However, the data reported in genome-wide linkage studies, candidate gene association studies, and, more recently, genome-wide association studies do not support a substantial role of single

genes in the development of asthma.Future research should be planned to explore gene–environment and gene–gene interactions to unravel the etiology of this complex condition.

2 The Genetic Bases of Asthma

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KEY POINTS/TAKE-HOME MESSAGES

- Numerous genetic, environmental, and hormonal factors underlie the pathogenesis of asthma leading to an abnormal antigen–antibody reaction inducing a vago-vagal axon reflex-mediated bronchoconstriction.
- Asthma has a complex and largely undefined genetic background. Similar to most complex diseases, its heterogeneous phenotype is thought to result from the interaction between multiple genes and environmental factors.
- It has been estimated that 73% of asthma determinants are genetic. Moreover, genetic variation is also thought to account for approximately 60–80% of the inter-individual variability in therapeutic response to medical treatments.
- Epigenetics seems to explain the corticosteroid resistance in patients with COPD.
- Over 25 genes have been hypothesized to be involved in asthma pathogenesis. However, the data reported in genome-wide linkage studies, candidate gene association studies, and, more recently, genome-wide association studies do not support a substantial role of single genes in the development of asthma.
- Future research should be planned to explore gene–environment and gene–gene interactions to unravel the etiology of this complex condition.

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INTRODUCTION

[AU1]

Asthma is characterized by acute clinical symptoms such as bronchoconstriction, bronchial inflammation and cough due to increased smooth muscle contractility, epithelial secretion, and tissue remodeling ultimately leading to airway thickening. Numerous genetic, environmental, and hormonal factors underlie the pathological antigen–antibody reaction inducing a vago-vagal axon reflex. The biochemical mechanisms involved in asthma exacerbations include a disequilibrium between two opposite second messenger systems in the airways, favoring the PLC–PKC over the cAMP cascade.

Asthma has a complex and largely undefined genetic background. Similar to most complex diseases, its heterogeneous phenotype is thought to result from the interaction between multiple genes and environmental factors. The concordance of asthma in monozygotic twins is 65%, significantly higher compared to 25% in dizygotic twins *(1)*, thus supporting a necessary but not sufficient genetic component in the individual predisposition to the disease and it has been calculated that 73% of asthma determinants are genetic. Moreover, genetic variation is also thought to account for approximately 60–80% of the inter-individual variability in therapeutic response to medical treatments. One of the major obstacles in these studies is represented by the wide clinical variability of asthma in terms of severity, age of onset, confounding factors (such as tobacco smoke), and treatment response. Asthma is probably the most common chronic disease in children of developed country; then, significant effort has been invested in the search for its genetic predisposition factors. Unfortunately, there has been independent replication only for a few study findings, mostly due to the lack of statistical power, differences in study design, and demographic differences of the studied populations resulting in different genetic background or environmental factor exposure *(2, 3)*. Genome-wide linkage studies, candidate gene association studies, and, more recently, genome-wide association studies (GWAS) have been used to investigate the genetic basis of asthma over the past decades. Several chromosomal regions were found to be linked to asthma and related disorders, and a number of genes within these regions seem to be biologically relevant in the pathogenesis of the disease.

In general terms, the proteins encoded by the reported genes are mostly transcription factors, and cytokines or receptors involved in Th2 polarization, chemokines and chemokine receptors playing a role in inflammatory cell recruitment into the airways, chloride channels responsible for hypersecretion, proteins regulating allergic reaction through IgE, leukotrienes, prostaglandins, and major histocompatibility proteins or proteins involved in reactive oxygen species scavenging and tissue remodeling (Table 1). These findings will be critically discussed in the present chapter and we will illustrate the possible functional implications of the reported associations, while further details on the pathogenesis and treatment issues can be found in other chapters of this book.

THE CASE OF ATOPIC DERMATITIS

The first atopic manifestation is thought to be atopic dermatitis, being the skin the first site of sensitization, thus making dermatitis an ideal companion for asthma in this discussion. Generally, during the first years of life, a progression from atopic dermatitis to asthma and allergic rhinitis develops and no individual risk factor is sufficient to explain

Table 1
Major Genetic Associations Reported in Asthma and the Relative Protein Function

Gene	*Chromosome*	*Protein function*
IFNG	12q14	Cytokine involved in Th1 response
STAT6	12q13	Transcriptional factor regulating Th2 response
IL-4R	16p12	Cytokine receptor involved in Th2 response
IL-13	5q31	Cytokine involved in Th2 response
ADAM33	20p13	A disintegrin and metalloprotease (ADAM) metallopeptidase domain 33
GPRA	7p14	Neuropeptide S receptor 1
DPP10	2q14	Dipeptidyl-peptidase
PHF11	13q14	PHD finger protein 11
CD14	5q31	Myeloid cell-specific leucine-rich glycoprotein
TLR4	9q33	Protein recognizing pathogen-associated molecular patterns (PAMPs) that are expressed on infectious agents (i.e., bacterial LPS)
TLR2	4q32	Protein recognizing PAMPs that are expressed on infectious agents (i.e., gram-positive bacteria and yeast)
TLR9	3p21	Protein recognizing unmethylated CpG dinucleotides in bacterial DNA
CARD15	16q21	Caspase-recruitment domain containing protein 15 (CARD15), cytosolic receptor involved in bacterial recognition by antigen-presenting cells
TGFB1	19q13	Cytokine involved in fibrogenesis
ADRB2	5q31	β-2-Adrenergic receptor
NOS	12q24	Nitric oxide synthase
SPINK5	5q32	Serine peptidase inhibitor, Kazal type 5
GST (T1,M1,P1)	22q11,1p13 11q13	Glutathione *S*-transferases (GST)
PDE4D	5q12	Lung-expressed phosphodiesterase implicated in airway contractility
ORMDL3	17q21	Transmembrane protein anchored to endoplasmic reticulum
GSDML	17q21	Gasdermin protein regulating apoptosis in epithelial cells
DENND1B	1q31	Protein interacting with TNF-α receptor and expressed by natural killer and dendritic cells

these alterations in global atopic disease prevalence *(4)*. Differences in the prevalence between urban and rural populations or farming communities have been attributed to the risk of atopy, including diet, hygiene, infections, allergens, and air pollution, in combination with genetic factors. Exposure to household pets, livestock, unpasteurized milk, and endotoxins during childhood are associated with a reduced incidence of allergic manifestations, although the data are inconsistently reported overall *(4)*. Nevertheless, there is convincing evidence that demonstrates a clear correlation between increased microbial

exposure and reduced allergic sensitization. In recent studies comparing geographically distinct but genetically related pediatric populations in Finland and Russia, a significant, dose-dependent reduction in the risk of atopy associated with microbial cell content and prevalence of enteroviruses has been demonstrated *(5–7)*.

The mechanisms by which allergen exposure through the epidermis could initiate systemic allergy and start the so-called "atopic march" *(4)* and predispose individuals to asthma have been elucidated during the past years. There is evidence implicating a primary inherited epithelial barrier defect resulting from *filaggrin* gene null mutations as a major predisposing factor in a subset of patients with atopic dermatitis and, secondarily, to the development of asthma *(8)*. Other less known epithelial defects, such as [AU2] *SPINK5*, also may have a role *(9)*. Proinflammatory factors derived from keratinocytes and other epithelial cells have also elicited considerable interest, including thymic stromal lymphopoietin, which has been shown to stimulate mast cells to produce Th2 cytokines *(10)*. These data suggest potential molecular targets for preventing allergen sensitization associated with epithelial barrier disruption and halting the progression of atopic dermatitis and other atopic diseases.

GENOME-WIDE LINKAGE STUDIES

Prior to the GWAS era, several genome-wide linkage studies or candidate gene association studies were performed to identify gene and chromosomal regions linked to asthma. Nevertheless and as previously mentioned, differences in study design, studied population origins, low statistical power led to various results. Genome-wide linkage studies reported that the regions and genes more consistently associated with asthma are cytokine cluster on chromosome 5q, *INFG* (INF-γ) and *STAT6* on 12q, and *IL-4R* [AU3] (IL-4Rα) on 16p *(3)*.

Serum INF-γ levels have been found to be significantly lower in patients with atopic asthma. IFN-γ plays an important anti-inflammatory role in asthma, as it suppresses tumor necrosis factor (TNF)-α signaling in atopic patients, and expression of IL-6, IL-8, and eotaxin induced by exposure to TNF-α. It also induces inflammatory genes such as vascular endothelial growth factor (VEGF), and the expression of IL-17 receptor *(11)*. The increased acetylation of the nuclear factor κB (NF-κB) p65 subunit as a result of TNF-α signaling is considerably reduced by IFN-γ. These findings suggest that IFN-γ suppresses the expression of some, but not all, pro-inflammatory genes induced by TNF-α by interfering with the transcriptional activity of NF-κB, possibly through changes in acetylation levels of the key regulatory proteins. Based on this background, the single nucleotide polymorphism (SNPs), T–A, at the 5′ end of the CA repeat of the human *IFN*-γ gene (+874T/A) directly affects the level of IFN-γ production and the A874 allele correlates with a low production of IFN-γ *(4)*. This polymorphism seems to coincide with a putative NF-κB binding site that could have functional consequences for the transcription of the human *IFN*-γ gene, with the result that the polymorphism could directly influence the level of IFN-γ production.

STAT6 is critical for Th2 cytokine signaling *(12)*. Multiple sequence variants of the *STAT6* gene have been identified, some of which are associated with atopic phenotypes in diverse populations *(12)*. Seven dinucleotide GT repeat variants were identified in the noncoding exon 1 of *STAT6*. Case–control association analysis of 214 white British

subjects demonstrated significant association with asthma of an allele with a 13GT repeat sequence (GT13), whereas the GT16 allele showed an inverse association with asthma. Furthermore, individuals with the GT13 allele had a higher level of IgE compared with individuals with the GT16 allele. Transient transfection assays of different alleles revealed significantly higher transcriptional activity with the GT13 allele compared with the GT16 allele in in vitro cell lines. Moreover, the GT13 allele had significantly decreased binding stability compared with the GT16 allele in a reciprocal competitive assay. These findings suggest that the GT repeat polymorphism of the *STAT6* gene contributes to susceptibility to atopic asthma and total serum IgE levels, and that variation in the length of the GT repeat sequence influences the regulation of promoter activity *(12)*.

Linkage studies followed by positional cloning have identified novel genes involved in asthma susceptibility including *ADAM33* on chromosome 20p, *GPRA* on chromosome 7p, *DPP10* on chromosome 2q, and *PHF11* on chromosome 13.

ADAM33 is a member of the ADAM (a disintegrin and metalloprotease) family. ADAM proteins are involved in cell adhesion, cell fusion, cell signaling, and proteolysis *(13)*. These proteins have the capacity to shed cytokines, growth factors, or their receptors from the cell surface and the remodeling of extracellular matrix components. The enzymatic activity of ADAM33 can be inhibited by tissue inhibitor of metalloproteinase-3 and -4 (TIMP-3 and -4, respectively) as well as several small molecules. This suggests that ADAM33 is involved in pulmonary defenses and tissue remodeling. In fact, a crucial pathological feature of chronic respiratory diseases such as asthma is airway inflammation and remodeling leading to airflow obstruction. A truncated, soluble form of ADAM33 containing the catalytic domain caused rapid induction of endothelial cell differentiation in vitro and angiogenesis ex vivo and in vivo, thus suggesting its possible involvement even in lung vascular remodeling in COPD. Genome-wide screening revealed that chromosome 20p13 was significantly linked to asthma and airway hyperresponsiveness in 460 families with asthma from the UK and the USA *(14)*. This genomic region contains the gene *ADAM33*. Since the first report of an association between *ADAM33* polymorphisms and asthma in two Caucasian populations from the UK and the USA, a number of replication studies have been published with differing results *(14)*. The differences in the association results may be due to phenotypic and environmental heterogeneity between cohorts. Additional studies demonstrated that SNPs within the *ADAM33* locus are associated with accelerated decline of lung function in the general population and in patients with asthma. The *ADAM33* gene is expressed in airway smooth muscle cells and fibroblasts in the lung, suggesting that it is not only important in the development of asthma but also in disease progression, possibly through airway remodeling *(13)*. These latter findings suggest a function of *ADAM33* related to lung growth and repair in general rather than solely associated with asthma. Recent studies revealed that SNPs within *ADAM33* confer susceptibility to COPD in the general population and are associated with airway inflammation in COPD.

GPRA, G-protein-coupled receptor for asthma susceptibility, also known as G-protein-coupled receptor 154, *GPR154*, located on chromosome 7 was identified as an asthma candidate gene by positional cloning in Finnish and French Canadian populations *(15)*. *GPRA* has two main isoforms with alternatively spliced 3′ exons (371 amino acids for isoform A and 377 amino acids for isoform B) and distinct tissue distribution

patterns. Expression patterns of the GPRA-B pulmonary isoform are different between asthma patients and healthy controls *(15)*. Moreover, levels of the GPRA-B isoform are increased in airway smooth muscle cells and epithelial cells in asthma patients compared to healthy controls. These data suggest that GPRA plays a role in asthma pathogenesis. *GPRA* single nucleotide polymorphisms (SNPs) and haplotypes have been associated with asthma or atopy in several studies, but not in others. In the studies in which associations have been found, however, the SNPs and haplotypes related to asthma and atopy are inconsistent across populations. Thus, the role of genetic variation in *GPRA* in asthma and atopy remains inconclusive.

CANDIDATE GENE ASSOCIATION STUDIES

Over the past years, candidate gene association studies identified several candidate genes with a few of these results being replicated in subsequent works.

Some of the candidate genes are involved in innate immunity such as TLRs, *CD14*, *CARD15*. The development of allergic disease may be influenced by bacterial and viral infections *(2)*. Thus, genes involved with the innate immunity response are obvious candidates for the understanding of the protective effects of exposure to microbial agents on allergy and asthma. Indeed, several SNPs in genes encoding pattern recognition receptors such as *CD14* and toll-like receptors (TLR) have been associated with atopic sensitization and asthma. Gene–environment interactions were found between ten SNPs in *CD14*, *TLR4*, *TLR2*, and *TLR9* and living in the country during childhood, which was presumed to represent higher exposures to various microbial agents *(3)*. Of note, these observations follow the hygiene hypothesis that has been proposed for numerous immune-mediated conditions *(3)*. Main effects and gene–environment interactions were stronger in subjects who were atopic than in those who were nonatopic. In particular, an association has been found between the *TLR2/+596* polymorphism and asthma and between *CD14/−260* SNP and asthma.

Since TLR2 is involved in the recognition of microbial motifs of a wide range of Gram-positive microorganisms, mycobacteria, and yeast, the exposure to these microorganisms is likely to occur more frequently in rural compared to industrialized areas. A lower expression of TLR2 on the surface of innate immune cells in carriers of the *TLR2/+596C* allele would be associated with a lesser protective effect of environmental exposures to TLR2 ligands on asthma *(16, 17)*. On the other hand, TLR9 is a receptor for bacterial CpG DNA motifs and the studies investigating *TLR9* SNPs in relation to allergy or asthma have reported inconsistent associations. However, significant gene–gene interactions with the *TLR2/+596* SNP were demonstrated, showing effects of two *TLR9* SNPs on asthma in *TLR2/+596 TT* subjects *(16, 17)*. Interestingly, TLR9 and TLR2 have different ligands and these observations may cumulatively reflect an interactive effect of multiple microbial exposures to determine asthma onset.

Genetic variants of the caspase-recruitment domain containing protein 15 (CARD15) that might result in inappropriate immunomodulation are not only associated with autoimmune diseases *(18)*, but also with atopic disorders. CARD15 is a cytosolic receptor involved in bacterial recognition by antigen-presenting cells. Subjects carrying the T allele at rs1077861 manifest a decreased risk of developing asthma, whereas the

presence of an A allele at rs3135500 is significantly associated with an increased risk *(19)*. In addition, a *CARD15* haplotype revealed to be protective against the development of asthma *(19)*.

Other candidate genes involved in inflammation include specific cytokines and chemokines, and also the respective signaling pathway such as mediators involved in IL-4/IL-13 signaling. IL-4 and IL-13 are pleiotropic, proinflammatory cytokines produced by activated T cells as part of an immune response to allergen exposure. The genes for IL-4 and IL-13 lie in a cytokine cluster on chromosome 5q31, a locus previously linked to several asthma phenotype. IL-4 and IL-13 are characterized by structural and functional similarities, as well as a common receptor component, IL-4Rα, located on chromosome 16p11. IL-4 plays important roles in T cell development, eosinophilic inflammation, and IgM–IgE isotype switching in B cells. IL-13 is a Th2 cytokine found to be overexpressed in the lungs of patients with asthma and in murine models of the disease *(20)*. Studies of the 5q31 locus reported significant associations between genetic variants in *IL-4* and *IL-13* genes and asthma or asthma-related phenotypes in some populations *(20–22)*. It has been reported an association between the *IL-4* C-589T allele and asthma severity in whites but not in African-Americans. Similarly, other nine SNPs in the *IL-4* gene have been found to be significantly associated with asthma or total serum IgE in whites *(21)*. It has also been reported an association between asthma-related phenotypes and polymorphisms in both the *IL-13* and *IL-4Rα* genes, as well as evidence of gene–gene interaction between *IL-13* C-1112T and *IL-4Rα* C+22656T (S478P) SNPs as a contributor to asthma susceptibility. In another study, a significant gene–gene interactions has been reported between the *IL-13* R130Q and *IL-4Rα* Ile50Val (A+4679G) polymorphisms for asthma risk in a Chinese population. Moreover, a significant gene–gene interaction was found between the *IL-13* (A-646G) and *IL-4Rα* (A-4679G) SNPs for baseline lung function among African-American subjects with asthma *(22)*. [AU4]

Interestingly, additional candidate genes are involved in lung function, growth, and development such as *TGFB1*, *ADRB2*, *NOS1* and *3*, and *SPINK5*. Polymorphisms in the transforming growth factor-β1 (TGF-β1) gene have been implicated in susceptibility to asthma, but a large number of studies have reported inconclusive results. A meta-analysis performed to investigate the association between polymorphisms in the *TGF-β1* gene and asthma susceptibility suggested that the −509C/T polymorphism in the *TGF-β1* gene may be a risk factor for asthma *(23)*. On the other hand, β-2-adrenergic receptors (β(2)AR) participate in the physiologic responses of the lung, including bronchodilation and bronchial protection, through mechanisms such as ciliary clearance, fluid accumulation, and mediator release from mast cells and basophils. Thus, these receptors may also play an important role in the pathophysiology of asthma. The gene encoding β(2)AR, *ADRB2*, is extremely polymorphic, but it appears that, for asthma, *ADRB2* polymorphisms are not etiologically involved *(24)*. However, they might affect disease severity and clinical response to both acute and chronic administration of β(2)-agonists. Finally, genes involved in the response to environmental exposures to pollutants and tobacco have been also found to be associated with asthma such as *GSTM1*, *GSTP1*, and *GSTT1*. Oxidative stress in the lungs has been implicated in the pathogenesis of asthma. Sources of oxidant injury are reactive oxygen and nitrogen species generated by activated [AU5]

inflammatory cells and bronchial epithelial cells and inhalation of atmospheric pollutants, notably tobacco smoke and oxidant gases, including ozone, sulfur dioxide, and nitrogen oxides. These are countered by enzymatic and nonenzymatic antioxidants, including dietary antioxidants, such as flavanols, vitamins C and E, and glutathione, a major protective antioxidant in the lungs that also has a role in regulation of inflammatory responses. The enzyme family of glutathione-*S*-transferases (GST) has the general function of conjugating glutathione with electrophilic substances that are capable of generating free radicals, thus leading to detoxification of their effects. Genetic polymorphisms associated with reduced activity of GST are therefore of interest in the study of asthma susceptibility. Two common deletion polymorphisms of *GSTM1* and *GSTT1* genes have been associated with asthma in children and adults *(25)*. The Val allele of the *GSTP1* Ile105Val polymorphism, associated with reduced glutathione activity, has been reported to be either protective or associated with increased risk of asthma. A recent meta-analysis does not support a substantial role of *GST* genes on asthma phenotypes in either children or adults, although small effects cannot be excluded and it is possible that these genes act on airway disease through interaction with environmental exposures or other genes *(25)*. Future studies on larger populations are warranted to evaluate *GST* genes in addition to other antioxidant genes or to air pollution and tobacco smoke exposures or the possible association of *GST* genes with asthma severity are needed to provide evidence on gene–gene interactive effects on asthma.

One final and fascinating hint for the etiology of asthma comes from a rare condition. The mutation of *SPINK5* causes Netherton syndrome, a rare recessive skin disease that is accompanied by severe atopic manifestations including atopic dermatitis, allergic rhinitis, high serum IgE, hypereosinophilia, and asthma. The SNP −206G>A of the *SPINK5* promoter is significantly associated with atopy, atopic dermatitis, asthma, and total serum IgE *(26)*. Moreover, the A allele at −206G>A has a significantly higher transcriptional activity than the G allele. Electrophoresis mobility shift assay also showed a significantly higher binding efficiency of nuclear protein to the A allele compared with the G allele.

GENOME-WIDE ASSOCIATION STUDIES

GWAS involving large cohorts of patients and controls have recently been performed in a growing number of complex diseases and, more specifically, identified novel asthma-associated gene regions. The first GWAS in 2007 identified several markers on chromosome 17q21 specifically associated with nonatopic childhood-onset asthma *(27)*. The study examined over 317,000 SNPs in 994 patients and 1,243 controls from UK and Germany, and, subsequently the results have been confirmed in Northern Europeans, North Americans of European ancestry, Puerto Ricans, Mexicans, Japanese, and Chinese, but not in African-Americans *(3)*. Combining gene expression levels with the associated SNP genotype a significant asthma association was found with the transcripts of *ORMDL3*, a transmembrane protein anchored to endoplasmic reticulum, and of *GSDML*, a gasdermin protein regulating apoptosis in epithelial cells. Another GWAS included 359 North American of European ancestry asthmatic patients and demonstrated a significant association between asthma and variants of the *PDE4D* gene, mapped on chromosome 5q12 and coding for a lung-expressed phosphodiesterase

involved in airway contractility *(28)*. Ten independent studies attempted to replicate this association, but only in Caucasian cohorts a weak association between asthma and two out of seven *PDE4D* SNPs was reported *(3)*. Further, a recent GWAS, together with the previously reported association with 17q21 locus, demonstrated a novel asthma locus on 1q31 in two independent cohorts of 793 and 917 patients with asthma of North American of European ancestry, but not in African ancestry patients *(29)*. The locus contains the gene *DENND1B* which is expressed by natural killer (NK) and dendritic cells and is possibly involved in the TNF-α pathway. Interestingly, in the patients of African ancestry 17 SNPs at 1q31 locus have been found to be associated with asthma but at each SNPs the alternative allele was associated with asthma compared to the discovery set. Finally, a GWAS has been conducted on 935 African-American, 929 African Caribbean, but no significant associations were determined *(30)*.

EPIGENETICS

The incomplete concordance between monozygotic twins and the reported associations observed in subgroups of patients with asthma clearly suggest that additional factors are needed to determine disease onset. Accordingly, epigenetics (i.e., DNA methylation and/or various post-translational modifications of histones mediated by acetyltransferase/deacetylase enzymes) appears as an ideal link between the environment and genomics and may thus play an important role in the expression of multiple inflammatory genes in asthma *(31)*. Moreover, epigenetics seems to explain the corticosteroid resistance in patients with COPD.

Gene expression is determined by a balance between histone acetylation which activates transcription and deacetylation which switches off transcription. An altered expression of inflammatory genes and an elevated acetylation of histone-4 were found in patients with asthma; moreover, the degree of histone acetylation seems to correlate with disease severity *(32)*. It has been observed that in the lung tissue of patients with asthma the increased acetylation of histones associated with inflammatory gene hyperexpression is not secondary to an increase in histone acetyltransferase activity, but due to decreased histone deacetylase activity. These mechanisms are particularly interesting in consideration of the fact that the anti-inflammatory activity of corticosteroids is partly due to epigenetic mechanisms and is directed to suppress NF-κB regulated genes including several of the inflammatory genes hyperexpressed in asthma *(31)*. After diffusing across cell membrane, corticosteroids bind their receptor and translocate into the nucleus where the receptor has to be acetylated to bind the glucocorticoid receptor recognition element sited in the promoters of the steroid-sensitive genes. However, it is also necessary that the corticosteroid receptor is deacetylated by histone deacetylase 2 (HDAC2) to inhibit NF-κB. In peripheral blood mononuclear cells and alveolar macrophages of patients with asthma and corticosteroid resistance, HDAC2 has been found to be markedly reduced *(32)*. Further, the corticosteroid resistance of COPD bronchoalveolar macrophages is reversed by overexpression of HDAC2. The mechanisms resulting in HDAC2 reduction in COPD are based on the inactivation, ubiquitination, and degradation of the enzyme by oxidative and nitric oxide-mediated stress. The formation of peroxynitrite, which nitrates tyrosine residues on HDCA2, and the activation of PI3k-δ by oxidative stress, which leads to phosphorylation of HDCA2, are the main

mechanisms underlying corticosteroid resistance in COPD. In addition, corticosteroids switch on corticosteroid-responsive genes, such as MKP-1, via acetylation of K5 and K16 on histone-4, and it has been reported, in some asthma patients, that acetylation of histone-4 K5 fails to occur. Besides the role of histone acetylation and deacetylation in the regulation of inflammatory genes, histone methylation seems also to be involved; moreover, corticosteroids seem partially act through this mechanism in inhibiting inflammatory genes *(33)*.

Interestingly, it has been proved that theophylline can selective activate HDCA2 in macrophages of COPD patients, and ultimately counteract and reverse corticosteroids resistance. These data have been reproduced in murine models and in smokers asthma patients. Theophylline is effective in accelerating COPD exacerbation recovery and to reduce inflammatory mediators. The mechanism of action of theophylline at molecular level seems to be via inhibition of PI3κ-δ *(34)*.

CONCLUSIONS AND FUTURE DIRECTIONS

This summary of the observed genetic associations in asthma clearly illustrate our incomplete understanding of the susceptibility to this complex condition. The mechanisms involved in the genetics of asthma are illustrated in Fig. 1. Similar to other multifactorial diseases, GWAS were welcomed as the solution to our knowledge gaps but have so far failed to prove conclusive or to report associations that may be used in the clinical workup of patients or first-degree relatives of patients. Indeed, reported associations include potential candidate genes that fit well within the current pathogenesis theories. On the other hand, we submit that the upcoming availability and accessibility of next-generation sequencing and genome-wide epigenetics tools may report additional associations (possibly with rare variants) or complementary mechanisms for transcription regulation. Further, given the potential for interactions between the genes found to be asthma-related and environmental toxins known to cause oxidative damage to the lungs, future research should be planned to explore gene–environment interactions. Large studies with accurate measurement of the environmental exposure are needed in order to reach adequate power to detect such interactions. Failure to account for environmental exposures might partly explain not only the heterogeneity of results across studies, but also the overall negative findings. Strong environmental effects on asthma phenotypes could mask modest genetic effects and, more importantly, gene–environment interactions could make the effects of genes become substantial only in the presence of oxidative exposures and not detectable at a population level. Passive smoking, ambient air pollution and endotoxin or other pathogen-associated molecules are good candidates for gene–environment interactions in asthma. Variation in exposure to these environmental factors across studies is likely to have happened given the diverse geographical setting of the studies included, and gene–environment interactions might partly explain the large heterogeneity observed. Moreover, there is evidence that antioxidant supplementation can modify these gene–environment interactive effects, so that the nutritional status of the study population could represent an additional source of heterogeneity. The evaluation of gene–environment interactions is problematic due to the lack of power of statistical tests for interactions and the

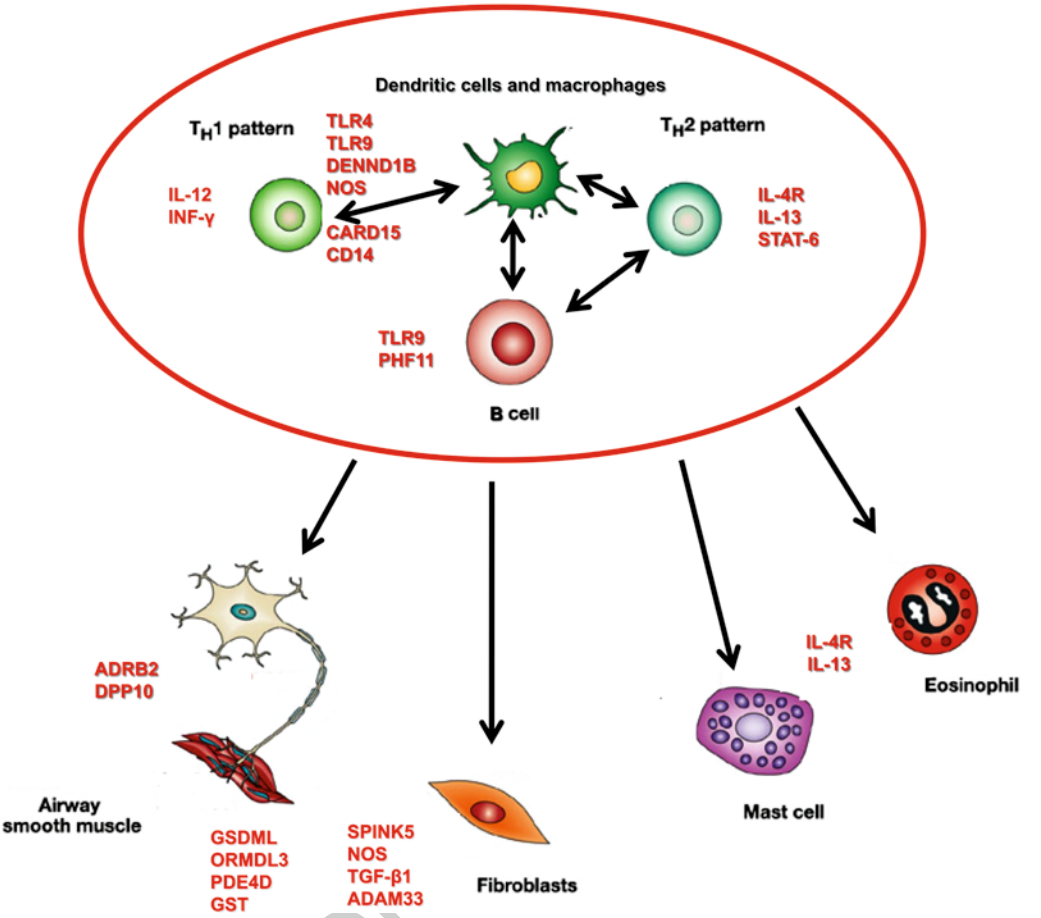

Fig. 1. The functional role of the majority of asthma-associated genes in the pathogenesis of the disease.

high measurement error present in the assessment of most environmental exposures. In fact, despite the strong biological rationale, results from the literature on gene–environment interactions in asthma remain inconclusive. Standardization of methods for environmental exposure assessment and full reporting of the interactions tested will allow the pooling of data across studies and to reach adequate power to detect interactions.

Similarly, further research should evaluate possible gene–gene interactions. Moreover, the contribution of ethnicity, childhood *vs.* adult asthma, and age at onset should be considered. Differences in asthma definition may also have played a role in generating the observed heterogeneity. Asthma diagnosed by a physician, self-reported doctor-diagnosed asthma, and self-reported history of asthma differ in sensitivity and specificity. Moreover, asthmatic individuals identified through questionnaire in a population-based study may have lower severity than patients recruited at a clinic.

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Author Queries

Chapter No.: 2 0001286611

Queries	Details Required	Author's Response
AU1	Please italicize gene names in the chapter.	
AU2	Term "Spink5" has been changed to "SPINK5". Please check if appropriate.	
AU3	Term "IL-4Ra" has been changed to "IL-4Rα". Please check if appropriate.	
AU4	Please approve amended text here	
AU5	Please check whether "TGFB1" can be changed to "TGF-β1".	