

Diversity of asthma: Evolving concepts of pathophysiology and lessons from genetics

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Asthma is a complex respiratory disorder characterized by reversible airflow obstruction and bronchial hyperreactivity. In recent years, our understanding of the mechanisms underlying allergic asthma has grown, and this has resulted in complex paradigms to define the immunobiology of the disorder. These paradigms conceive of asthma as a disorder with complex genetic and environmental interactions that affect the developing immune system and ultimately result in the episodic release of procontractile mediators, including leukotrienes and prostaglandins, causing susceptible individuals to wheeze. Genetic studies seek to advance our knowledge by identifying the differences in our genetic make-up that cause us to be susceptible. Surprisingly few of the verified asthma genes involve known asthma mediators. This article reviews several of the best-established asthma genes, including a disintegrin and metalloprotease 33, dipeptidyl peptidase 10, PHD finger protein 11 (PHF11), and the prostanoid DP1 receptor. Identification of these unsuspected genes is leading to models of asthma pathogenesis that go beyond our conception of asthma as a disease of smooth muscle abnormalities, inflammatory cell presence, and airway structural changes. (*J Allergy Clin Immunol* 2005;115:S526-31.)

Key words: *Asthma, genetics, disease mechanisms, leukotriene, prostaglandin*

ASTHMA PREVALENCE AND EPIDEMIOLOGY

Asthma is now epidemic in the developed world and might affect as many as 1 in 4 urban children.¹ It is associated with substantial morbidity, mortality, and economic cost.² Asthma and allergy are increasingly common in westernized countries, including the United States.^{3,4} Asthma-associated morbidity, disability, or both affect 54 of every 1000 Americans and are now thought to affect 17 million Americans.⁵ In 2001, 8 of every 10 persons with asthma in the United States visited an office-based physician, and 2.8 million visited an emergency department⁶ when primary controller treatment failed.^{7,8} Bronchial hyperresponsiveness is the physiologic hallmark of asthma but also occurs in individuals without asthma and can be found in 10% to 15% of the general population.

Abbreviations used

ADAM: A disintegrin and metalloprotease
CCR: C-C chemokine receptor
DPP-10: Dipeptidyl peptidase 10
LT: Leukotriene
PGE₂: Prostaglandin
PHF11: PHD finger protein 11

BIOLOGY OF ASTHMA

The rapid growth in our understanding of allergic mechanisms has led to new and more complex explanatory paradigms for asthma.⁹⁻¹¹ We now conceive of asthma as a disease of gene-environment interaction with complex immunobiology. Allergic asthma is thought to be initiated early in life by the airway delivery of allergen. The first step in the immune recognition of asthma is antigen entry into immune cells. Antigen-presenting cells, including dendritic cells, take up foreign proteins that can serve as antigens, hydrolyze them into short polypeptides, and cause them to be expressed on the cell surface in the binding groove of MHC II. Antigen presentation by MHC II on the surface of antigen-presenting cells is associated with the surface expression of accessory molecules that facilitate apposition of the loaded MHC II-binding groove to the T-cell receptor. Antigen uptake by antigen-presenting cells is thought to occur near the airway surface and is followed by migration of these cells, which bear the C-C chemokine receptor 7 (CCR7) to regional lymphoid tissue under the influence of the CCR7 ligands, EBI-1 ligand, and secondary lymphoid tissue chemokine, which also attract CCR7-bearing naive and central memory T lymphocytes. In the regional lymphoid tissue, which includes bronchial associated lymphoid tissue and bronchial and hilar lymph nodes, accessory molecule ligand-counterligand pairs allow activation of the T-cell receptor by the antigen-loaded MHC II antigen-presenting cell. Local cytokine expression is thought to have profound effects on the response of T lymphocytes to antigen presentation in a process referred to as immune deviation. In the presence of IL-12, these cells have an IFN- γ -expressing or T_H1-type phenotype. Impaired ability to express the T_H1-type phenotype might be important in asthma because mice with a genetic defect that prevents T lymphocytes from deviating down the T_H1 pathway have an asthma-like phenotype.¹² Under the influence of IL-10, these cells can develop the T-regulatory cell phenotype that might be important in limiting progression to asthma. It is the influence of IL-4 and IL-13 that leads to the development

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of the T_H2 phenotype that appears to be required for expression of the asthma phenotype. Importantly, the combined action of IL-4, IL-13, CD40, and an enzyme called activation-induced cytidine deaminase induces deletion switch recombination in B lymphocytes and the production of allergen-specific IgE, levels of which are increased in atopic asthma. It is the ability of IgE to specifically bind allergen and receptors on the effector cells of the allergic response, including mast cells, basophils, and eosinophils, that allows release of the mediators that cause asthma symptoms.

EFFECTOR MOLECULES IN ASTHMA

The recurrent and episodic nature of asthma exacerbations cannot be explained by fixed structural changes in the airway. This observation has led to concepts of asthma that include the episodic release and subsequent metabolism of effector molecules, including histamine, platelet-activating factor, proteases, and arachidonic acid metabolites. Prostaglandins and leukotrienes are metabolites of arachidonic acid that are produced enzymatically during allergic and asthmatic reactions and are present at restricted sites within the airway microenvironment and influence lung resident cells by acting at specific receptors.¹³ Many of the inflammatory cell types that are associated with asthma and allergic diseases, including mast cells, basophils, and eosinophils, produce leukotrienes. In addition to production by inflammatory cells, structural cells within the lung can produce prostaglandins.¹⁴

LEUKOTRIENES IN ASTHMA

Cysteinyl leukotrienes are produced from arachidonic acid liberated from the sn-2 position of perinuclear membrane triglycerides by the action of cytosolic phospholipase A2. Molecular oxygen is added to the sn-5-6 position of arachidonic acid through the action of the enzyme 5-lipoxygenase in the presence of its molecular chaperone, 5-lipoxygenase activating protein. This 2-step reaction that results in the formation of the unstable epoxide leukotriene A_4 (LTA_4) is followed by the addition of the tripeptide glutathione in cell types expressing LTC_4 synthase. LTC_4 thus generated is transported out of the cell and degraded by means of sequential amino acid removal to LTD_4 and the less potent LTE_4 , which is the primary urinary metabolite. The cysteinyl leukotrienes act at the cysteinyl leukotriene 1 and 2 receptors.¹³

The cysteinyl leukotrienes are the first class of effector molecules demonstrated in clinical trials to promote the asthma phenotype. Part of the increased baseline tone present in the asthmatic airway is due to the activity of the 5-lipoxygenase enzyme and the actions of the cysteinyl leukotriene receptor. The finding that asthma exacerbation rates are reduced by leukotriene modifiers indicates that exacerbations are dependent in part on the activity of 5-lipoxygenase¹⁵ and the action of the cysteinyl leukotriene receptor.¹⁶

Differences in transcriptional efficiency can be accounted for by differences in DNA binding protein affinity

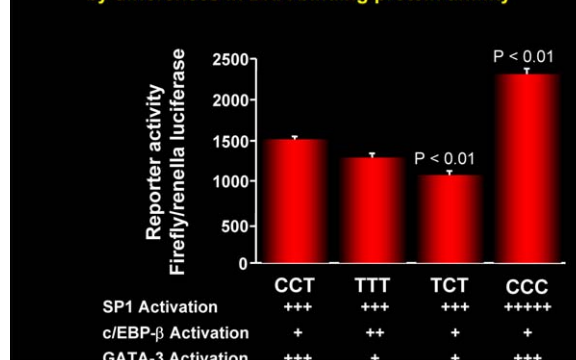


FIG 1. Differences in transcriptional efficiency can be accounted for by differences in DNA-binding protein affinity. *SP1*, specificity protein 1; *c/EBP-β*, cyclic AMP responsive element binding protein.

There is emerging evidence that the action of prostaglandins and leukotrienes in the airways has the potential to influence inflammatory cell recruitment and alter the activation state of mesenchymal cells and thus influence airway remodeling.¹⁴ Asthma-associated damage to the airway epithelium might limit production of substances, such as prostaglandin E2 (PGE_2) and 15-hydroxy peroxy eicosatetraenoic acid (HETE), that might limit mesenchymal repair responses.

PGE_2 AND PGD_2 IN ASTHMA

The availability of genetically altered mice has provided new insights into the role of these prostaglandins in the airway.¹⁷ Among prostanoid receptor-deficient mice, those deficient in the D-prostanoid (DP) receptor have the most profound alteration of allergic phenotype. DP receptor-deficient mice were sensitized to an intraperitoneal injection of 50 μ g of ovalbumin and 1 mg of alum on days 0 and 12 and inhalation of 10 mg/mL nebulized ovalbumin on days 22, 26, and 30. Compared with DP-sufficient mice, DP-deficient mice had reduced levels of T_H2 cytokines and eosinophils in their bronchoalveolar lavage fluid and did not have airway hyperresponsiveness in a methacholine model.¹⁸ After allergen challenge, DP receptor expression was increased in ciliated and non-ciliated airway epithelial cells and in type II alveolar epithelial cells. PGD_2 is an intriguing effector molecule because its major source is mast cells, which are activated after allergen exposure and are increased in the asthmatic airway, including near airway smooth muscle. In addition to animal studies, there are human studies implying that the *PTGDR* gene that encodes the DP receptor for PGD_2 is associated with susceptibility to asthma. The *PTGDR* gene is located on a region of chromosome 14 that has been associated with asthma and atopy. The core promoter region of this gene contains combinations of genetic variants that differ with respect to their ability to support receptor transcription. Individuals who bear low

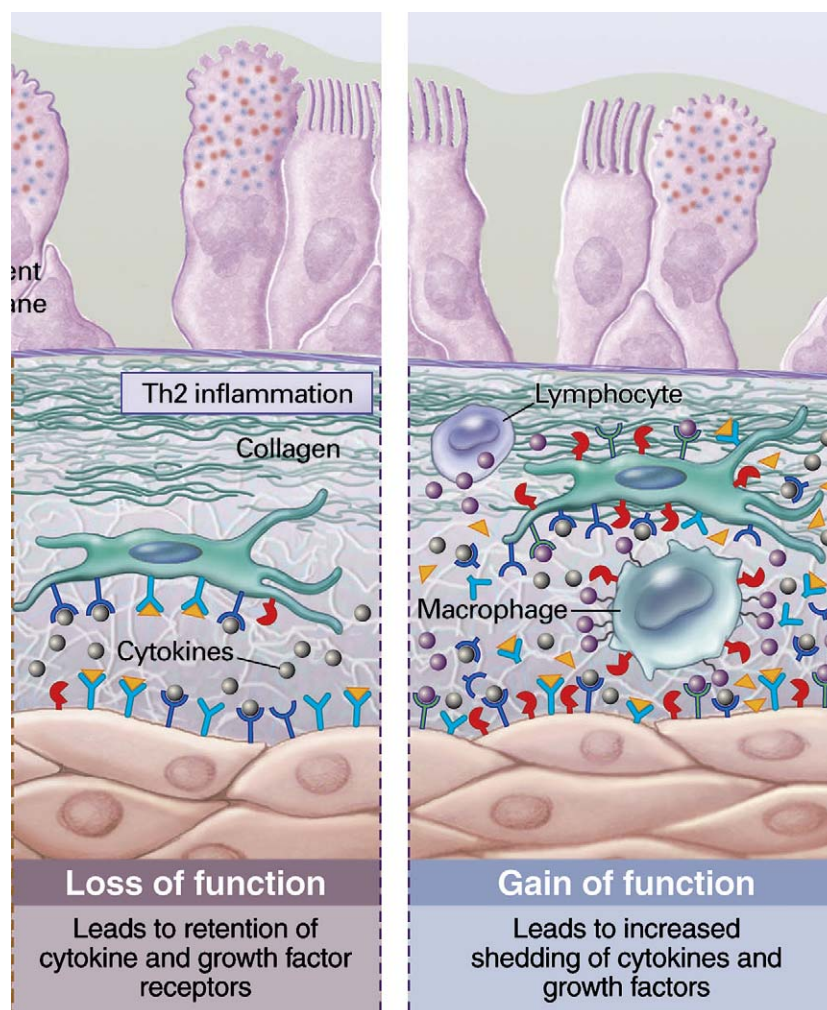


FIG 2. ADAM33 and asthma. Adapted with permission from Shapiro and Owen. N Engl J Med 2002.²⁴

transcriptional efficiency variants are at lower asthma risk, and those bearing higher transcriptional efficiency are at higher risk than individuals having the more common forms of the gene (Fig 1).

The airway effects of PGE₂ have been clarified by studies of mice deficient in the 4 forms of the E-prostanoid (EP) receptor.^{19,20} The effects of PGE₂ on airway tone vary significantly among both mouse strains and among human subjects. In addition to bronchoconstriction, its airway administration can be associated with retrosternal burning and cough. Insight into these differences has been provided by studies of aerosol PGE₂ administration to mice deficient in the EP receptor. In systems in which neuronal influences are suppressed or absent, PGE₂ can induce bronchodilation that is dependent on the EP2 receptor. PGE₂ bronchoconstriction is dependent on intact neural function and on the EP1 receptor and, in some strains of mice, the EP3 receptor. These observations are important because they imply that an EP2-specific agonist might be useful as a reliever in asthma.

ISOPROSTANES

In addition to being formed by means of enzymatic modification of arachidonic acid, prostaglandins can be formed as a result of oxidative stress. The activation of peroxidase-containing airway granulocytes is associated with the generation of airway oxidants and, in the presence of halides, hypohalous acids. When the formation of reactive oxygen and nitrogen species exceeds the reducing capacity of the airway microenvironment, the peroxidation of membrane lipids can occur. The realization that this process can result in the formation of isoprostanes had led to interest in using stable isoforms as measures of airway oxidative stress. The characteristics of 8-isoPGF₂α have created interest in this molecule as a surrogate measure for the peroxidation of airway membrane lipids. Its presence can be detected in airway lining fluid and in condensate of exhaled gas by means of gas chromatography mass spectrometry or by means of enzyme immunoassay. Its concentration is increased in asthmatic subjects compared

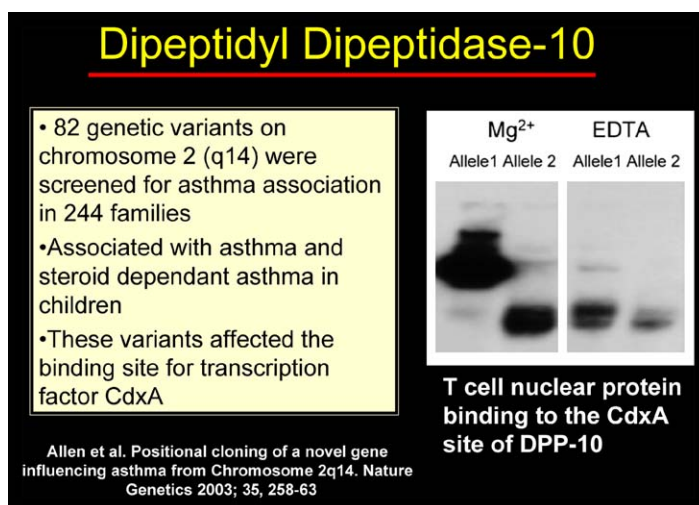


FIG 3. *DPP10*. Reprinted with permission from Allen et al. © 2003 *Nature Genetics*.²⁵

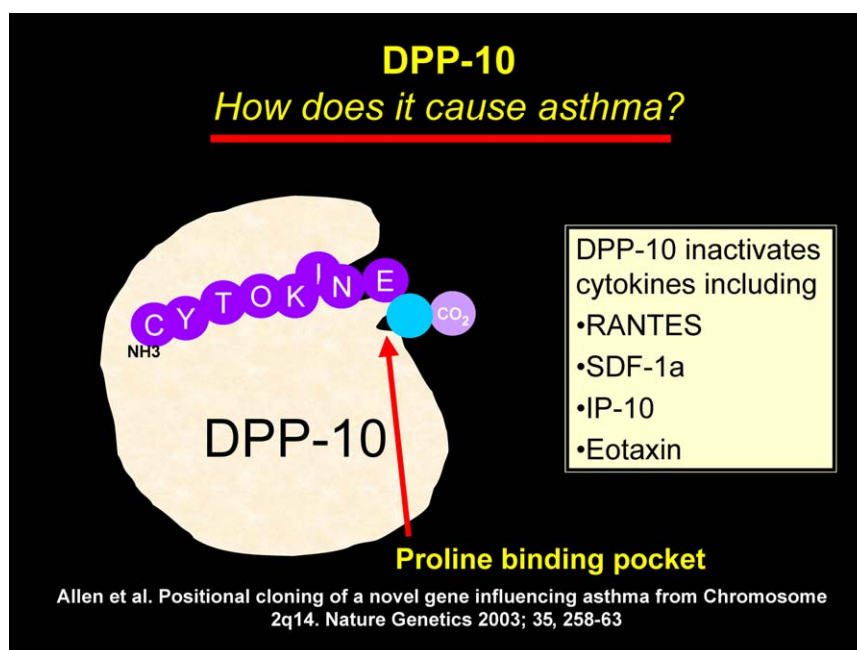


FIG 4. *DPP10*: How does it cause asthma? *SDF-1a*, stromal-cell derived factor-1a; *IP-10*, interferon inducible protein 10. Reprinted with permission from Allen et al. Copyright © 2003 *Nature Genetics*.²⁵

with levels in healthy subjects, but there is substantial overlap.²¹ Like measurements of nitric oxide, nitrogen species metabolites, airway lining fluid pH, and cytokines in condensed exhaled gas, this noninvasive measurement awaits longitudinal study and definition of its predictive power in asthma.

ASTHMA GENES IDENTIFIED BY MEANS OF GENETIC ASSOCIATION

The availability of polymorphic markers that span the human genome and well-phenotyped families with mem-

bers with asthma has allowed the identification of genetic variants that are associated with asthma and its related phenotypes. In examining populations from 12 studies, the genes suspected to play an important role in asthma (ie, *IL4*, *IL13*, *T-bet*, and *GATA3*) did not correlate with the disease. Instead, the genes that have been associated with asthma by using these methods have not been involved in known asthma pathways. These include a multifunctional gene known as a disintegrin and metalloprotease 33 (*ADAM33*),²² dipeptidyl peptidase 10 (*DPP10*),²² and PHD finger protein 11 (*PHF11*).²³ The mechanisms by which the variants of these genes influence asthma pathogenesis is an area of active investigation but likely

involves effects on multiple determinants of airway cell growth and differentiation by influencing the expression of regulatory ligands and receptors.²²

The first asthma-associated gene was identified by using studies of asthma association of specific variants in regions of the genome associated with asthma by means of genome-wide screening. The *ADAM33* gene is located on the short arm of chromosome 20 and is a member of the metalloproteinase subfamily of enzymes, which hydrolyze peptide bonds of other proteins with an active center that includes a zinc atom. There are approximately 35 *ADAM* genes described that are distinguished by the presence of both a metalloproteinase and disintegrin domain. They were originally described as cell-surface proteins that were important for egg-sperm fusion, and many, like *ADAM33*, are present and active on the surface of cells, whereas other family members that contain a thrombospondin domain can be secreted. *ADAM* genes are not only important for cell fusion and proteolysis but also for cell adhesion, signaling, and the hydrolytic activation and release of cytokines, including TNF- α , from the surface of cells. It is thought that *ADAM33* might lead to asthma susceptibility and bronchial hyperresponsiveness by altering the release of proallergic cytokines or by inactivating the cell-surface receptors that transduce their responses (Fig 2).²⁴

The *DPP10* gene, located on the long arm of chromosome 2 at 2p14, has been associated with asthma by using positional cloning techniques (Fig 3).²⁵ *DPP10* encodes a member of the dipeptidyl peptidase family of proteins, which act to limit the activity of proteins, including cytokines, chemokines, and leukotrienes, that are released into the extracellular space. *DPP10* has substrate specificity for many cytokines that have been implicated as involved in promoting expression of the asthma phenotype, including RANTES, eotaxin, and macrophage chemotactic protein 2. Similar to the interpretations of the implications of the identification of *ADAM33*, the mechanistic link to asthma appears to be a gene product that can act on more than one proallergic cytokine or chemokine (Fig 4).²⁵

The same group that identified the *DPP10* gene has also associated the *PHF11* gene on chromosome 13q14 as being associated with asthma and increased levels of IgE.²³ Like the *ADAM-33* and *DPP-10* gene products, the *PHF11* gene product has the ability to affect many other mediators. The *PHF-11* gene product is a zinc-finger protein that is present in B lymphocytes and can interact with DNA to influence the expression of other gene products.

ASTHMA ASSOCIATION ESTABLISHED BY MEANS OF EXPRESSION GENOMICS

Measurement of the expression of large numbers of individual genes is now possible with expression array technology. This allows comparison of the genes that are expressed in the airways of asthmatic subjects, assessment of the effects of allergen on gene expression, and com-

parison of the expression of the human genome in cells from asthmatic subjects and healthy individuals. We have recently performed expression genomics studies before and after the application of clinically relevant concentrations of standardized allergen solutions to the epithelial surface of the lower airways of individuals with spirometrically mild asthma. We identified 141 sequences as having significantly increased expression and 8 that had significantly decreased expression. We identified several genes that are known to be associated with allergen exposure, including lipocortin, the p65 subunit of *NFKB*, and the *IL3*, *IL4*, and *IL5* receptor subunits. The largest fraction of identified genes were known to be involved in growth, differentiation, and proliferation. These results imply that asthma is more than a disease of airway inflammation and involves changes in the growth and tissue responses to injury that evolve over time.

AN EMERGING MODEL OF ASTHMA PATHOGENESIS

These insights into asthma pathogenesis have caused us to update our former models of asthma pathogenesis that focused on airway smooth muscle function and airway inflammatory pathways to incorporate concepts of regulatory systems that affect the expression of allergic cytokines and chemokines, including the effects of allergen on airway resident cell growth and proliferation,¹⁴ and this explains the role of the prostanoid DP receptor in asthma. In this model allergen, toxin, or irritant exposure not only leads to deviation of the adaptive immune system and recruits T_H2-type lymphocytes, eosinophils, and mast cells to the airway but also acts indirectly to position mast cells near airway smooth muscle cells²⁶ and activates the prostanoid DP receptor to promote their survival.²⁷ Repeated allergen exposure leads to changes in the activation state of airway epithelial cells and the release of factors that cause the transformation of subepithelial fibroblasts into the collagen-secreting myofibroblast phenotype. Deposition of collagen below the airway basement membrane alters the predicted response to constriction of airway smooth muscle from that of concentric narrowing to plication, deformation, and compromise of the luminal area.²⁸ It is the episodic release of procontractile mediators from airway eosinophils and mast cells that more efficiently causes the airway narrowing that results in asthma symptoms and exacerbations. Our emerging concept of asthma is as a disease of sensitivity to environmental toxins, allergens, and infectious agents that over time results in changes in airway cellular composition and structure.

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