

Advances in asthma, allergy mechanisms, and genetics in 2006

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This review discusses the main advances in animal models of allergic airway disease and genetics of asthma and allergy published in the *Journal* in 2006. This work highlighted and extended what has become the central dogma of allergic pathogenesis by highlighting the mechanisms involved in inducing a T_H2 response and in determining how T_H2 cytokines induce the allergic airway disease phenotype. By so doing, they have identified a considerable number of potential therapeutic targets. Genetic analyses, on the other hand, revealed novel, potentially important candidate genes, confirmed known ones, and refined our understanding of the putative role played by others, sometimes positively, sometimes negatively. These data reiterate allergic inflammation is a classic complex genetic disease—that is, a disorder in which multiple and distinct genetic determinants variously interact with one another and with relevant environmental exposures to result in clinical phenotypes that, although superficially similar, involve distinct genetic pathways and represent the outcome of distinct pathogenetic mechanisms. (*J Allergy Clin Immunol* ■■■■;■■■;■■■-■■■.)

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The *Journal* published 23 papers during 2006 that dealt with animal models of allergic airway disease (AAD); 22 of these used mouse models, and most used these models to evaluate AAD pathogenesis and/or potential therapy. Many of these studies enlarged on the concept that allergens induce CD4⁺ T cells and later other cells to secrete IL-4. IL-4, by activating signal transducer and activator of transcription (STAT)-6, promotes T-cell secretion of all T_H2 cytokines, including IL-13, the major effector cytokine in mouse models of AAD. In addition to

Abbreviations used

AAD:	Allergic airway disease
ADAMTS12:	A disintegrin and metalloproteinase domain with thrombospondin type 1 motif 12
AHR:	Airway hyperresponsiveness
AOAH:	Acyloxyacyl hydroxylase
BHR:	Bronchial hyperresponsiveness
DC:	Dendritic cell
ESR:	Estrogen receptor
IL-7R:	IL-7 receptor
LIFR:	Leukemia inhibitory factor receptor
PTGDR:	Prostanoid DP receptor
PTGER4:	Prostaglandin E4 receptor
SNP:	Single nucleotide polymorphism
STAT:	Signal transducer and activator of transcription
TLR:	Toll-like receptor
VEGF:	Vascular endothelial growth factor
ZFR:	Zinc finger RNA binding protein

its effects on macrophages and dendritic cells (DCs), IL-13 acts on smooth muscle cells, epithelial cells, vascular endothelial cells, fibroblasts, and nerve cells through STAT6 to promote airway hyperresponsiveness (AHR), goblet cell hyperplasia, chemokine production, inflammatory cell infiltration, and increased vascular permeability.

Several articles published in the *Journal* during 2006 added details of how IL-13 promotes AAD. IL-13-induced vascular endothelial growth factor (VEGF) was shown to signal through PI3K δ to increase vascular permeability, and a PI3K δ antagonist was shown to reduce inflammatory cell infiltration, T_H2 cytokine secretion, AHR, vascular permeability, and the production of VEGF itself.¹ Pulmonary VEGF expression and angiogenesis in a murine AAD were also shown to be suppressed by treatment with a Toll-like receptor (TLR)-9 ligand, possibly by reducing pulmonary macrophage number, inhibiting macrophage expression of VEGF, and suppressing expression of IL-13.² Treatment with a TLR9 ligand also suppressed expression of matrix metalloproteinase 9, which is up-regulated in a mouse model of AAD and is thought to contribute to airway remodeling in asthma.³ T_H2 cytokines (along with TNF and IL-1 β) were shown to stimulate airway epithelial cells to produce neurotrophins that bind to specific receptors (tropomyosin-related kinase A and tropomyosin-related kinase B) on eosinophils; neurotrophin stimulation of eosinophils was required for the

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increased survival of eosinophils cultured with airway epithelial cells and may promote pulmonary eosinophil survival.⁴ Neurotrophins were also shown to contribute to AAD in other ways: Trk receptors are upregulated in neurons, airway smooth muscle, and peribronchial inflammatory cells after allergen challenge in a murine AAD, and a Trk receptor antagonist decreased sensory nerve hyperactivity and IL-4 and IL-5 levels but not smooth muscle hyperactivity, IL-13 levels, or bronchoalveolar lavage cell numbers in this disorder.⁵

Inhaled IL-4 was shown to stimulate both AHR and goblet cell hyperplasia,⁶ which are IL-13-dependent in murine AAD. This suggests that the IL-13 dependence of these characteristics reflects greater production or potency of IL-13 than IL-4 in this disorder, rather than a unique IL-13 signaling pathway, and is consistent with the possibility that dual IL-4/IL-13 antagonists may be more efficacious than pure IL-13 antagonists in some individuals with asthma. Both IL-4 and IL-13 were also shown to induce TGF- β -independent secretion of periostin by lung fibroblasts. Periostin was shown to bind to other extracellular matrix components involved in subepithelial fibrosis in human asthma and murine AAD and may contribute to development of the subepithelial fibrosis that is a characteristic of remodeling in these disorders.⁷

IL-4 promotion of T_H2 differentiation may also be responsible for an observation that sensitization with 1 allergen can enhance systemic and airway T_H2 responses to a second allergen. Consequently, suppression of IL-4 responses by early immunotherapy may inhibit sensitization spreading.⁸ Similarly, an observation that epicutaneous exposure to *Aspergillus fumigatus* antigen primes mice for development of allergic upper airway disease in response to intranasal inoculation with *A. fumigatus* antigen may depend on an IL-4 response to that antigen, inasmuch as development of the allergic upper airway disease was STAT6-dependent.⁹ Both IL-4 and IL-13 may be effector cytokines in allergic upper airway disease. IL-13 was required for the late-phase but not the early-phase increase in nasal resistance in a mouse model of allergic rhinitis and contributed little to eosinophilia or IgE responses¹⁰ (IL-4 contributes more than IL-13 to both IgE induction and airway eosinophilia in the mouse).

Other cytokines may promote AAD indirectly by promoting production of IL-4 and/or IL-13. IL-9 and IL-25 (also called IL-17E) are prominent in this group. Pulmonary IL-25 expression is increased in murine AAD, and an IL-25 antagonist decreased pulmonary CD4⁺ T-cell numbers and eosinophilia. Transgenic pulmonary IL-25 overexpression enhanced airway eosinophilia, T_H2 cytokine production, and goblet cell hyperplasia in a CD4⁺ T-cell-dependent, STAT6-dependent mouse model of asthma, although it had no effect on these parameters in the absence of antigen administration.¹¹

Additional articles published in the Journal during 2006 used mouse models to evaluate therapeutic approaches to prevent and suppress AAD, at least in part by decreasing pulmonary T_H2 cytokine production. These approaches included (1) administration of either of 2 serine protease

antagonists, nafamostat mesilate and gabexate mesilate, which reduced mast cell activation, AHR, and eosinophil infiltration and promoted a shift away from T_H2 and proinflammatory cytokines and toward T_H1 and anti-inflammatory cytokines¹²; (2) biolistic injection of allergen (which uses a high speed jet of air to cause allergen particles to penetrate a surface) into skin or buccal mucosa, which inhibited IgE and eosinophil responses to subsequent ovalbumin inhalation without stimulating a T_H1 response¹³; (3) administration of agonists for peroxisome proliferator activated receptors (ubiquitously expressed transcription factors that, when activated, protect against oxidative stress) or an adenovirus that carries peroxisome proliferator activated receptor- γ cDNA. Both treatments reduced AHR and decreased reactive oxygen species generation and expression of T_H2 cytokines, chemokines, VEGF, and the transcription factors nuclear factor- κ B and hypoxia-inducible factor 1 α ¹⁴; (4) administration of a bispecific antibody fragment that cross-links the chemokine receptor CCR3 with CD300a (a molecule that inhibits eosinophil and mast cell activation), which suppressed mast cell and eosinophil activation and established airway inflammation, T_H2 cytokine secretion, and remodeling¹⁵; and (5) administration of adiponectin, an insulin-sensitizing adipokine that is reduced in obese individuals, who have an increased incidence of asthma. Adiponectin and adiponectin receptor levels were reduced in murine AAD, and adiponectin treatment reduced AHR, T_H2 cytokine production, and airway inflammation.¹⁶

A third group of papers, although less focused on specific therapies for AAD, also contributed to the understanding of AAD pathogenesis and treatment. One demonstrated that CD4⁺ T-cell expression of the signaling protein inducible T-cell (tyrosine) kinase (ITK) contributes to the generation of a T_H2 cytokine response in AAD.¹⁷ A second suggested that the chemokine (C-C motif) ligand 21 (CCL21), which promotes entry of naive T cells and antigen-stimulated DCs into T-cell zones of secondary lymphoid organs, contributes to both the development of AAD and its resolution (apparently by promoting regulatory T-cell responses)¹⁸; consequently, CCL21 antagonists may not be preferred agents for suppressing AAD. A third paper identified a novel possible target for suppressing airway remodeling in AAD by demonstrating that increases in gene expression and secretion of the TGF- β family member, activin A, correlated better with human asthma severity than increases in TGF- β 1 and that activin A, but not TGF- β 1, was induced in a mouse model of AAD. Activin A was shown to induce TGF- β 1 and may link acute T-cell activation in asthma with chronic TGF- β 1-induced airway remodeling¹⁹; however, studies with activin A antagonists are required to test this possibility. Finally, a study that used oral or nasal allergen administration to induce antigen-specific tolerance in an ovalbumin model of murine AAD showed that suppression of AHR, goblet cell hyperplasia, and eosinophilia was easier to accomplish than suppression of IgE production, and suppression of IgG1 production was still more difficult.²⁰ This observation should be useful for planning

TABLE I. Key advances in asthma, allergy mechanisms, and genetics in 2006

1. Treatment with TLR9 ligands suppresses multiple pulmonary allergic responses, including VEGF-dependent increased vascular permeability and matrix metalloprotein 9-dependent airway remodeling.
2. IL-4 and IL-13 can independently induce both airway hyperresponsiveness and goblet cell hyperplasia.
3. Sensitization with 1 allergen can enhance systemic and airway T_H2 responses to a second allergen.
4. Endogenously produced IL-25 can contribute to T_H2 cytokine production and allergic inflammation.
5. Allergic inflammation and remodeling can be suppressed by cross-linking CCR3 with the inhibitory receptor CD300a.
6. Administration of the insulin-sensitizing adipokine, adiponectin, reduces AHR, allergic airway inflammation, and T_H2 cytokine production.
7. A single nucleotide polymorphism in the coding region of *IL17F* results in the expression of a natural IL-17 antagonist inversely associated with asthma.
8. The combined analysis of genetic alterations within a pathway provides better insights into the biological significance of genetic variants.
9. Filaggrin null mutations are strongly associated with eczema and concomitant asthma and predispose to asthma, allergic rhinitis, and allergic sensitization, but only in the presence of eczema.
10. The genetic basis for the allergic response to seasonal allergens involves both substantial genetic overlap and extensive heterogeneity.
11. A broad region on chromosome 5p, separated by >9 Mb, harbors at least 2 and possibly 5 susceptibility loci for asthma or BHR, suggesting that regions providing evidence for linkage in multiple populations may house more than 1 susceptibility locus.

therapeutic trials of antigen-specific immunotherapies and evaluating the results of such trials.

In sum, mouse model studies of AAD supplemented what has become the central dogma of AAD pathogenesis by fleshing out the mechanisms involved in inducing a T_H2 response and in determining how T_H2 cytokines induce the AAD phenotype (Table I). By so doing, they have identified a considerable number of potential targets for AAD treatment.

FROM GENES TO PHENOTYPES AND BACK: 1 YEAR OF ASTHMA/ALLERGY GENETICS IN THE JOURNAL

As recently discussed in these pages,²¹ allergic reactions involve multiple cellular and molecular interactions that ultimately converge to activate common effector mechanisms of disease. In addition to the classic players such as the T_H2 inflammatory pathway, experimental allergy and clinical studies keep highlighting novel molecules and genes, each critical in the context of individual models. In the face of large numbers of studies and models, it is increasingly difficult to gauge the relative role old and new components play in the response of outbred human populations to natural allergens. Genetics may offer a way out of this maze in that strong and reproducible associations between genetic variants and allergy-related phenotypes eloquently confirm the role played by a given gene *in vivo*. Conversely, lack of genetic evidence should cast more than a shadow of a doubt on the involvement of that gene. During 2006, the Journal published more than 20 papers devoted to the genetics of allergy-related traits. Here we briefly discuss how these studies affirmed, confirmed, refined, or disproved the role specific genes and their products play in the pathogenesis of allergy and asthma (Fig 1).

The most upstream interactions in allergic inflammation occur at the innate sensing interface that involves allergens, microbial products, and DCs surveying the microenvironment.²¹ DCs, in turn, present antigen to CD4 T_H precursors under the watchful influence of T-

regulatory cells. In this scenario, it is not surprising a number of innate immunity and immunoregulatory genes are emerging as major determinants of susceptibility to allergy and/or asthma, often in the context of complex gene-environment interactions.²² The strong protective effects of farm exposure on the development of allergy-related traits provide a robust model to decipher the role of genetic components in modulating susceptibility to allergic inflammation. Focusing on 2 French centers participating in the European Community Respiratory Health Survey II, Leynaert et al²³ assessed whether the protective effects of farm exposure on atopy are influenced by variants in *CD14*, a major component of the TLR2/TLR4 signaling pathway. By administering a detailed questionnaire on farm exposure in childhood and genotyping a single nucleotide polymorphism (SNP), *CD14C*-159T, which results in increased *CD14* transcription, this group confirmed that exposure to a farming environment in early life is associated with a reduced risk of nasal allergies and atopic sensitization in adulthood. A lower risk of allergic rhinitis and atopy was observed in carriers of the *CD14*-159TT genotype. Interestingly, when farm exposure and *CD14* genotype were considered together, the risk of nasal allergies and atopy was most reduced in *CD14*-159T homozygotes exposed to a farming environment in early life, suggesting gene-environment interactions between *CD14C*-159T and farm exposure in childhood may modify the development of atopy.

In a variation on a similar theme, Williams et al²⁴ assessed whether levels of endotoxin (a product of Gram-negative bacteria and a ligand for TLR4/CD14), exposure to furred pets, and *CD14C*-260T (also known as *CD14C*-159T) interact to affect total serum IgE levels in adults. In this study, the *CD14C*-260T genotype was significantly associated with total IgE levels, but this relationship appeared to be modified by levels of endotoxin in the environment. At lower levels of endotoxin exposure, the CC genotype appeared to be associated with higher IgE levels when compared with the CT and TT genotypes, whereas at higher levels of endotoxin exposure, TT individuals had the highest IgE levels. Interestingly, interactions between *CD14C*-260T and

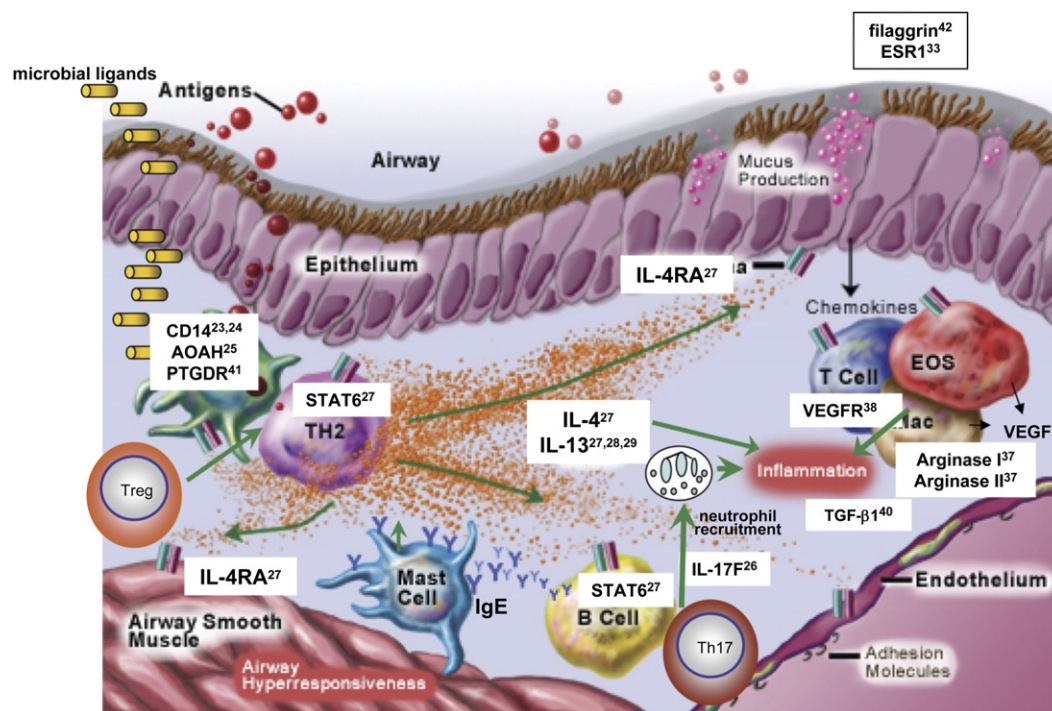


FIG 1. Genes critical for the pathogenesis of allergic inflammation. Individual pathways and genes highlighted by 2006 articles in the Journal are discussed in the text. References are numbered as in the text. *VEGFR*, VEGF receptor.

pet exposure were not apparent, suggesting separate mechanisms of action.

Still within the domain of innate immunity, the work by Barnes et al²⁵ illustrates how genetic association studies guided by the results of linkage analyses can identify novel disease candidate genes. The gene for acyl-coyl hydroxylase (*AOAH*), an enzyme that hydrolyzes secondary fatty acyl chains of LPS, is localized on chromosome 7p14-p12, a region known to be linked to total IgE concentrations and asthma. The Baltimore group hypothesized variants in *AOAH* may be a source of the linkage signal. Furthermore, they tested for *AOAH/CD14* interactions because both *AOAH* and *CD14* respond to LPS. Among 125 African Caribbean, multiplex asthmatic pedigrees, significant associations were detected between *AOAH* markers in 3 distinct regions of the *AOAH* locus and asthma, total IgE concentrations, the IL-13/IFN- γ ratio, and soluble CD14 levels. Comparing genotypic distributions at both the *AOAH* marker rs2727831 and *CD14C*-260T raised the possibility of gene-gene interactions. Although these studies need to be replicated and extended to analyze the impact of *AOAH* on innate immune functions, they also exemplify the ability of genetics to identify potentially important novel players in physiology and disease.

IL-17F is a recently discovered cytokine that plays a role in tissue inflammation by inducing release of proinflammatory and neutrophil-mobilizing cytokines. Upregulated *IL17F* expression has been observed at sites of allergen challenge in the airways of patients with asthma, suggesting IL-17F may be involved in asthma

pathogenesis. Kawaguchi et al²⁶ investigated the association between asthma and *IL17F* variants in a Japanese population using a powerful combination of genetic and functional studies. Five SNPs were studied, including a coding variant (*IL17FT*7488C, rs763780) resulting in a His/Arg substitution at amino acid 161. Homozygosity for the rare H161R variant was inversely associated with asthma. Of note, unlike wild-type IL-17F, IL-17F H161R failed to activate the mitogen-activated protein kinase pathway, cytokine production, and chemokine production in bronchial epithelial cells. Furthermore, IL-17 H161R blocked induction of IL-8 expression by wild-type IL-17F, indicating this variant is a natural IL-17F antagonist that influences the risk of asthma.

The effector phase of allergic inflammation involves multiple pathways and mechanisms orchestrated by the T_H2 cytokines IL-4 and IL-13 acting through their common signaling mediator, STAT6. The preeminent role of T_H2 cytokines is clearly highlighted by the work of Kabesch et al.²⁷ Rather than limit their analysis to single genes, this group assessed the combined effect on asthma and IgE levels of allelic variants arrayed along the T_H2 -dependent pathway (*IL4*, *IL13*, their shared IL-4 receptor α chain, *IL4RA*, and *STAT6*). This approach, although consonant with biology, decreased the statistical power of the analysis in spite of a large study population (1120 children age 9-11 years). However, the results remain striking. Combining polymorphisms in all 4 major genes in a stepwise procedure, the risk for high serum IgE levels increased by 10.8-fold and the risk for the development of

asthma increased by 16.8-fold compared with the maximum effect of any individual SNP. Significant interactions were observed in an additive and dominant model, indicating that the combined analyses of genetic alterations within a pathway provide better insights into the biological significance of genetic variants. Following a similar logic, Chan et al²⁸ investigated the interaction among 12 different loci in 8 candidate genes and asthma and increased plasma total IgE concentrations in Chinese children with asthma and control children. Their analysis revealed significant interactions between *IL13* and *IL4RA* for asthma, and *IL13* and the gene for thymus- and activation-regulated chemokine for total plasma IgE.

The critical role of genetic variants in *IL13*, a major effector of allergic inflammation, emerged also, perhaps unexpectedly, from a British study devised to assess an intriguing hypothesis. Atopic illnesses and type 1 diabetes, an autoimmune disease, have been reported to be inversely associated. One possible explanation is that susceptibility alleles for one disease provide protection for the other. Using the largest population sample reported so far for the identification of genetic determinants of circulating IgE levels (4570 DNA samples obtained from members of the British 1958 Birth Cohort), Maier et al²⁹ investigated associations between total serum IgE and SNPs in 8 genes that are candidate susceptibility loci for IgE levels/atopic illness (*IL13*, *IL4*, *IL4RA*, *FCER1B*, *IL12B*, *TBET*) and/or type 1 diabetes (*CTLA4*, *PTPN22*, *IL2RA*). The results provided no evidence of association of the confirmed type 1 diabetes susceptibility genes *CTLA4* and *PTPN22* and the candidate gene *IL2RA* with IgE levels. However, there was strong evidence of a positive association between *IL13* gene variants leading to increased *IL13* transcription and total serum IgE levels. Therefore, allelic variation in the *IL-13* gene was robustly confirmed as a contributor to the variance of IgE levels but had no detectable effect in type 1 diabetes. As extensively discussed in the editorial that accompanied this article,²² these results point to a complex counterregulation between T_H1 -mediated and T_H2 -mediated immunity. Moreover, they indirectly suggest that relevant environmental exposures may provide useful constraints for future genetic analyses of complex diseases like asthma/allergy and type I diabetes, which have major environmental determinants.

Along similar lines, work from several groups suggest the notion of what counts as an environment should be extended to include the milieu internal to the organism.^{30,31} Sex in particular appears to act as an environmental factor leading to differential effects of the same variant in men and women.³² In this context, Dijkstra et al³³ hypothesized that variants in the estrogen receptor (*ESR*) α gene, *ESR1*, may alter estrogen action in asthma and contribute to the higher prevalence and severity of adult asthma in women. This study identified several *ESR1* polymorphisms associated with development of bronchial hyperresponsiveness (BHR), particularly in female subjects, and/or with a more rapid loss of lung function in patients with asthma, specifically in women. These phenotypes may reflect altered estrogen action,

which affects lung development and/or airway remodeling.

Moving downstream along T_H2 -controlled effector pathways, genetic studies have corroborated 3 more candidate genes for allergy originally identified in animal models.³⁴⁻³⁶ Polymorphisms in arginase 1 and 2 were found to be associated with number of positive skin tests or increased relative risk of asthma, respectively, in a large Mexican population.³⁷ A strong association between an extended haplotype in VEGF receptor 2 and atopy prevalence was reported in a large cohort of Korean children and adolescents.³⁸

Replication across populations is a gold standard typically used to assess the true strength of a genetic association, because it implies a genetic effect may be robust enough to emerge in spite of distinct ethnic background and environmental conditions. Few genes evaluated so far (*IL13*, *IL4RA*, *FCER1B*, *ADRB2*, and *CD14* among them³⁹) have withstood the test of extensive replication. *TGFB1C-509T* is associated with asthma and atopy in white populations. Mak et al⁴⁰ used a case-control study to investigate the relationship between asthma and *TGFB1* SNPs in Hong Kong Chinese. Among atopic but not nonatopic subjects, significant differences were found in genotype and allele distribution for *TGFB1T869C* between patients with asthma and controls. Asthma risk was increased more than 2-fold in *TGFB1869C* homozygotes. Interestingly, risk for severe airflow obstruction was significantly increased in *TGFB1-509CT* heterozygotes, indicating that distinct *TGFB1* polymorphisms may contribute to distinct asthma subphenotypes.

When strong and well powered, negative studies can be quite instructive as well. A good example is provided by work on the prostanoid DP receptor (*PTGDR*) gene. *PTGDR*, a gene on chromosome 14q22.1, was recently identified as an asthma susceptibility gene. A haplotype with decreased transcription factor binding and transcription efficiency was found to be associated with decreased asthma susceptibility in African American and white subjects. Tsai et al⁴¹ tested the impact of *PTGDR* gene variants on asthma susceptibility and asthma-related traits in Latinos, the largest US minority population. This study, which took advantage of an in-depth characterization of *PTGDR* variants by resequencing, failed to identify significant associations between *PTGDR* variants and asthma among Puerto Ricans, Mexicans, or African Americans, reiterating that the marked genetic heterogeneity among human populations, potentially combined with differences in environmental exposures, likely results in the involvement of distinct pathogenetic pathways leading to asthma/allergy susceptibility.

Replication of genetic data, but only in selected respects, can also be quite informative. A major discovery in 2006 was that 2 loss-of-function mutations in the gene encoding the epidermal barrier protein filaggrin predispose to eczema and concomitant asthma. Because it is well known that childhood eczema often precedes the development of asthma and allergic rhinitis in what is often called the atopic march, these data raised the possibility the filaggrin

mutations might play a role in the transition from eczema to asthma. Marenholz et al⁴² assessed the effect of these mutations on the susceptibility to eczema and associated clinical phenotypes in 2 large European populations of children with eczema. The filaggrin null mutations were strongly associated with eczema and concomitant asthma and predisposed to asthma, allergic rhinitis, and allergic sensitization, but only in the presence of eczema. These results lend strong support to the role of filaggrin in the pathogenesis of eczema and in the subsequent progression of the atopic march. On the other hand, the fact that previous expression of eczema is a prerequisite for the manifestation of AAD and specific sensitization highlights the importance of the epidermal barrier in the pathogenesis of these disorders, suggesting that the maintenance and repair of the epidermal barrier in eczematous infants may prevent the subsequent development of AAD.

Although all of the studies discussed focused on candidate genes selected on the basis of biological plausibility and/or previous genetic evidence, the power of genetics lies also in its ability to discover genes potentially relevant to disease pathogenesis by performing wider scans relatively unconstrained by previous hypotheses. Two such studies were published in the Journal in 2006. The first one looked for genomic regions contributing to skin test reactivity to seasonal allergens, a major contributor to the asthmatic phenotype.⁴³ This study relied on a genome scan at 9-cM intervals on 287 families with 2 or more members with asthma and provided suggestive evidence for linkage to seasonal pollen reactivity for chromosomes 13q34, 20p12, and 21q21. Interestingly, ethnic differences in linkage signals were evident. Linkage was found on chromosomes 8, 10, and 12 in African Americans; chromosomes 14, 19, 20, and 22 in European Americans; and chromosome 21 in Hispanics. In all families, however, evidence for linkage of skin test reactivity for *Betula*, *Lolium*, and *Artemisia* was strongest in a region on chromosome 21 that contains the candidate gene a disintegrin and metalloproteinase 33 (*ADAM33*). These results suggest that the genetic basis for the allergic response to seasonal allergens involves both substantial genetic overlap and extensive heterogeneity.

The second study carries a different flavor because it relies on a combination of fine mapping and positional candidate studies. Because previous genome-wide linkage scans to identify asthma susceptibility loci had pointed to a broad segment of chromosome 5p, Kurz et al⁴⁴ genotyped 89 SNPs in 22 genes to identify a 5p-linked asthma or BHR locus using 2 distinct populations: Germans and Hutterites. Three genes in a distal region (zinc finger RNA binding protein [*ZFR*], natriuretic peptide receptor C, and a disintegrin and metalloproteinase domain with thrombospondin type 1 motif 12 [*ADAMTS12*]) were associated with BHR, whereas 4 genes in a proximal region (prolactin receptor, *IL7R*, leukemia inhibitory factor receptor [*LIFR*], and prostaglandin E4 receptor [*PTGER4*]) were associated with asthma symptoms in the Hutterites. Nearly the entire original linkage signal in this population was generated by individuals who had the risk-associated alleles in *ZFR3*,

natriuretic peptide receptor C, *ADAMTS12*, *LIFR*, and *PTGER4*. Variation in *ADAMTS12*, *IL7R*, and *PTGER4* was also associated with asthma in the German population, and the frequencies of long-range haplotypes composed of SNPs at *ZFR*, *ADAMTS12*, *IL7R*, *LIFR*, and *PTGER4* were significantly different between both the German and Hutterite cases and controls. Importantly, there is little linkage disequilibrium between alleles in these 2 regions in either population. These results suggest that a broad region on chromosome 5p, separated by >9 Mb, harbors at least 2 and possibly 5 asthma or BHR susceptibility loci. These findings are consistent with the hypothesis that regions providing evidence for linkage in multiple populations may in fact house more than 1 susceptibility locus.

In conclusion, the genetic analysis of asthma and asthma-associated traits published in the Journal in 2006 revealed novel, potentially important candidate genes, confirmed known ones, and refined our understanding of the putative role played by others, sometimes positively, sometimes negatively (Table I). Thus, the efforts of asthma/allergy geneticists were extremely productive. It may be argued no study so far succeeded in identifying the all-powerful, all-penetrant asthma gene, but the likelihood that such a gene exists is slim, because allergic inflammation more and more appears to provide a classroom paradigm for complex genetic diseases—disorders in which multiple and distinct genetic determinants variously interact with one another and with relevant environmental exposures to result in clinical phenotypes that, although superficially similar, involve distinct genetic pathways and represent the outcome of distinct pathogenetic mechanisms. Our hope is that the asthma genetics literature, in the Journal and elsewhere, will soon see the rise of novel, more powerful approaches better suited to capture the complexity of these interactions and their effect on clinical and biological phenotypes.

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