Advances in asthma and allergy genetics in 2007

Donata Vercelli, MD  Tucson, Ariz

This review discusses the main advances in the genetics of asthma and allergy published in the Journal in 2007. The association studies discussed herein addressed 3 main topics: the effect of the environment and gene-environment interactions on asthma/allergy susceptibility, the contribution of TLR2 immunity gene variants to allergic inflammation, and the role of filaggrin mutations in atopic dermatitis and associated phenotypes. Other articles revealed novel, potentially important candidate genes or confirmed known ones. Collectively, the works published in 2007 reiterate that allergy and asthma are typical complex diseases; that is, they are disorders in which intricate interactions among environmental and genetic factors modify disease susceptibility by altering the fundamental structural and functional properties of target organs at critical developmental windows. (J Allergy Clin Immunol 2008;122:267-71.)

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The year 2007 witnessed a significant strengthening of asthma and allergy genetics, and the Journal of Allergy and Clinical Immunology served as a premiere conduit for cutting-edge analyses of the genetic determinants of asthma/allergy susceptibility. Among the 26 genetics articles published in the Journal in 2007, some highlighted the remarkable effects of the environment and gene-environment interactions on immune functions and immune-mediated mechanisms of asthma and allergy, whereas others focused on variants in TLR2 inflammatory pathways. A third group of articles examined the effect on atopic dermatitis–associated phenotypes of variants in the filaggrin gene (FLG) (Table I). A fourth, more heterogeneous group of articles highlighted novel candidate genes or novel roles of known genes (outlined in Table II).1-14

ENVIRONMENT, GENES, AND GENE-ENVIRONMENT INTERACTIONS IN ASTHMA AND ALLERGY

Although it is now well established that being raised on a farm protects against hay fever and atopic sensitization, the evidence for an effect of farming on asthma and wheeze remains conflicting. Differences in farming practices and hence in microbial exposures might lead to discrepant results. Indeed, the multicenter Prevention of Allergy Risk Factors for Sensitization in Children Related to Farming and Anthroposophic Lifestyle (PARSIFAL) study recently revealed substantial heterogeneity in the protective ability of farming environments across study regions.15 It was therefore critical to assess whether distinct farm-related exposures have distinct effects on specific asthma-related phenotypes.

For their analysis, Ege et al16 relied again on the PARSIFAL study, which includes 8263 school-age children from rural areas in 5 European countries. A strong inverse relation with a lifetime diagnosis of asthma was found for pig keeping, farm milk consumption, frequent stay in animal sheds, child’s involvement in haying, and agriculture (the latter only in Germany). Use of silage strongly protected against nonatopic asthma. Interestingly, protective exposures correlated with higher expression of innate immunity genes (Toll-like receptors [TLRs] and CD14), and even more interestingly, distinct exposures correlated with increased expression of distinct genes; for example, haying was strongly related to increased TLR7 and TLR10 expression, whereas keeping pigs and feeding pressed hay were associated with higher levels of TLR5, and feeding silage was associated with increased TLR6 and TLR8 expression. Although the mechanisms responsible for the differential effects of exposure on distinct members of the TLR gene family remain unclear, it is tempting to speculate that these gene expression signatures point to the involvement of distinct microbial components in distinct ecologic niches within a complex farm environment.

Levels of endotoxin and extracellular polysaccharides were inversely related to atopic sensitization and asthma, respectively, independent of farm exposure. The protective effect of being a farm child on current wheeze was explained by the levels of exposure to endotoxin, glucans, and extracellular polysaccharides; however, these exposures did not explain the protective effect of farming on asthma and atopic sensitization. Also important was the identification of potential risk factors for asthma and wheeze, such as keeping hares and rabbits and the presence of sheep. Notably, after adjusting for the child’s

Abbreviations used

FLG: Filaggrin
LRTI: Lower respiratory tract infection
MYLK: Myosin light chain kinase
PARSIFAL: Prevention of Allergy Risk Factors for Sensitization in Children Related to Farming and Anthroposophic Lifestyle
RSV: Respiratory syncytial virus
SNP: Single nucleotide polymorphism
SPT: Skin prick test
TLR: Toll-like receptor
activities and farm characteristics, being a farm child was no longer inversely related with asthma, wheeze, or atopy. In other words, the variables included in the final models fully explained the effect of farming on asthma, wheeze, and atopy.

Collectively, these data point to complex biologic effects of farming on the immune and respiratory systems, a conclusion well attuned to the biologic complexity and heterogeneity of a farming environment. Most striking, though, was the demonstration that factoring in distinct and specific farm-associated exposures, activities, or both was sufficient to fully explain the protective effect of farming. This result implies that all the relevant variables have been identified, and adequate mechanistic models can now be devised to dissect the biology underlying the effect of farming on asthma, wheeze, and atopy.

One such model sought to define which farm microbial organisms, microbial products, or both might induce or influence allergy-protective mechanisms. Among a number of bacterial species identified in farm cowsheds, Debarry et al. selected, isolated, and characterized *Acinetobacter lwoffii* F78 and *Lactococcus lactis* G121. Both bacterial isolates upregulated costimulatory molecules and inflammatory cytokines and induced a TH1-polarizing program in dendritic cells *in vitro*. Moreover, intranasal administration of bacteria before and during ovalbumin sensitization reduced allergen-specific IgG1 (but not IgE) levels, eosinophil infiltration, peribronchial and peripheral inflammatory cell infiltration, mucus metaplasia, and airway hyperreactivity. The authors propose that the protective effects of these bacteria against allergic inflammation might be mediated by their TH1-promoting activity.

Work from the same European consortium also highlighted the contribution of gene-environment interactions to allergy susceptibility. Consumption of farm milk in early life is known to confer strong protection against asthma and allergies. Bieli et al. hypothesized that single nucleotide polymorphisms (SNPs) in *CD14*, the coreceptor for several TLRs, might modify the association between farm milk consumption and asthma and atopy. By investigating farmers’ and nonfarmers’ children from 2 European populations (Allergy and Endotoxin study, n = 576; PARSIFAL study, n = 1539), the authors detected a significant interaction between *CD14*−1721G homozygotes and farm milk consumption. Interestingly, the asthma-protective effects of farm milk consumption were dramatic for the AA genotype, less strong in *CD14*−1721G homozygotes, and undetectable in *CD14*−1721A homozygotes. Similar patterns were observed for allergic rhinoconjunctivitis and pollen sensitization. Importantly, *CD14*−1721 also modified the association between farm milk and *CD14* expression, which was significantly increased in −1721A homozygotes but not in carriers of the other −1721 genotypes. The authors conclude that the protective effects of farm milk consumption on allergic diseases might be mediated through farm milk–induced upregulation of *CD14* expression.

A different but equally intriguing example of gene-environment interactions affecting allergic inflammation susceptibility

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**TABLE I. Major advances in asthma and allergy genetics in 2007**

| 1. | Distinct exposures associated with farming affect the expression of distinct TLR family genes, pointing to the involvement of distinct microbial components in distinct ecologic niches within a complex farm environment. |
| 2. | The child’s activities and farm characteristics fully explain the effects of farming on asthma, wheeze, and atopy. |
| 3. | MYLK variants and haplotypes that confer risk for severe sepsis reduce the risk of allergic asthma in populations of African descent. |
| 4. | A functional *IL13* promoter polymorphism modifies the risk of asthma exacerbations and response to treatment. A functional *IL13* coding polymorphism is associated with late, but not early, wheezing after RSV infection. |
| 5. | The effect of *FLG* variants on the risk of atopic dermatitis exceeds that of any other candidate gene investigated thus far and makes *FLG* one of the strongest genes known to date for complex diseases. |

**TABLE II. Novel asthma/allergy candidate genes and novel roles of known genes**

| 1. | A *TNFA* promoter polymorphism (−308G/A) appears to be associated with severe bronchial hyperresponsiveness in Korean children with asthma, possibly in synergism with *CD14*−159CT. |
| 2. | An SNP in the FceRIα promoter appears to be associated with aspirin-intolerant chronic urticaria and increased gene expression in mast cells. |
| 3. | Leukotriene C4 synthase (*LTC4S*) −444AC is associated with IgE antibodies to *Dermatophagoides pteronyssinus* in a Colombian population. |
| 4. | Genetic variation in integrin *β3 (ITGB3)/CD61* affects asthma susceptibility and allergic sensitization, beginning early in life. Interestingly, different SNPs in the gene are associated with asthma and IgE. |
| 5. | Chronic *Mycoplasmata pneumoniae* infection appears to be associated with physician-diagnosed asthma and the defective chemokine (C-C motif) receptor 5 (*CCR5*) variant CCR5Δ32. |
| 6. | SNPs in S-nitrosoglutathione reductase (*GSNOR*) modulated asthma susceptibility in children from Mexico City. |
| 7. | A common mitochondrial haplogroup is associated with increased total serum IgE levels in white children participating in the Childhood Asthma Management Program. |
| 8. | *HLA-DRB1* alleles control allergic bronchopulmonary aspergillosis–like pulmonary responses in humanized transgenic mice. |
| 9. | SNPs in both the signaling lymphocytic activation molecule (*SLAM/CD150* and *CD46* genes are associated with measurable and significant variations in antibody response after measles vaccination. |
| 10. | Infant frequent wheezing is associated with Clara cell protein 10 (*CC10*) +38GA and lower CC10 levels, but not allergic sensitization, in a perinatal cohort study. |
| 11. | A common *IL31* haplotype is associated with increased *IL31* expression and nonatopic eczema. |
| 12. | Variants in chemokine (C-C motif) receptor 3 (*CCR3*) are associated with eosinophil counts, particularly in combination with IL-5 receptor α (*IL5RA*) polymorphisms. |
| 13. | *FCER2*, which encodes the low-affinity IgE receptor, predicts the likelihood of treatment success in asthmatic children. The associations of *FCER2*2206TC with IgE level, severe exacerbations, and *FCER2* expression might provide a mechanistic basis for these findings. |
| 14. | Polymorphisms in *IL4R* appear to be associated with Stevens-Johnson syndrome in Japanese patients. |
was provided by a study of myosin light chain kinase (MYLK), a multifunctional protein involved in the regulation of smooth muscle contraction and airway hyperreactivity.\textsuperscript{20} After showing that MYLK variants confer risk for sepsis and acute lung injury,\textsuperscript{21} the same group investigated the association between MYLK SNPs and asthma-related traits among African Caribbean and African American populations\textsuperscript{20} and compared findings from the asthmatic populations with findings in the African American sepsis and acute lung injury groups.

Significant associations between MYLK SNPs and asthma and total serum IgE concentrations were observed in the African Caribbean families: a promoter SNP (rs936170) in the smooth muscle form resulted in the strongest association. A haplotype including the same SNP significantly decreased asthma risk in both the American and Caribbean families. Interestingly, the same haplotype conferred risk for severe sepsis. RNA expression studies in peripheral blood monocytes pointed to a significant decrease in *MYLK* expression among asthmatic subjects who carry rs936170.

The authors note that several candidate genes for asthma and allergic diseases, such as *CD14, TLR4*, acetylcholyl hydroxylase, and now *MYLK*, are also associated with sepsis. These coassociations support the “common variant/multiple disease” hypothesis\textsuperscript{22} and underscore the pleiotropic effects of innate immunity genes in that variants conferring risk of sepsis under one set of genetic and environmental conditions reduce risk in a different (but possibly related) clinical setting (ie, allergic asthma).

Studies investigating the immunogenetics of asthma, atopy, or both have thus far only involved populations residing in the developed world.\textsuperscript{23} Given that populations can be genetically diverse, environmental conditions different, and causal pathways of allergic diseases not the same, the question is whether genetic associations found in the developed world also hold in the developing world, where the frequency of allergic diseases is low but increasing. Because parasite-induced IL-10 responses protected Gabonese schoolchildren from atopic reactivity,\textsuperscript{24} van den Bigge-laar et al hypothesized that human *IL10* variants that promote high IL-10 levels would be associated with reduced atopy in populations residing in Africa. To test their hypothesis, the authors genotyped 100 Gabonese schoolchildren with known skin prick test (SPT) reactions to house dust mite for 8 biallelic SNPs and 1 insertion/deletion polymorphism in the 5’-flanking region of *IL10*. Associations with SPT reactivity and PHA–induced IL-10 levels were investigated for individual polymorphisms and phased haplotypes. Although the results will need to be extended to a much larger population, these studies provided the first evidence that *IL10* variants promoting high IL-10 responses are associated with reduced risk for atopic reactivity in children living in Africa. Therefore in populations continuously exposed to infectious pathogens, the IL-10–mediated risk for atopy might be regulated both at an environmental (infection) and a genetic level. Considering the progressive urbanization of the developing world, it will be important to establish the relative role played by genetic and environmental mechanisms in the activation of IL-10 responses and the consequent risk for allergies and other inflammatory diseases in populations in transition.

**TH2 INFLAMMATORY PATHWAYS AND THEIR VARIANTS**

A second group of articles examined the effect of TH2 cytokine gene variants on susceptibility to allergic inflammation. The central effector role of IL-13 in this process is well established, and the effect of polymorphisms on the effector properties of IL-13 has also been highlighted by several studies.\textsuperscript{25} Two articles published in 2007 focused on the role of *IL13* variants in asthma and wheeze. Hunningshake et al\textsuperscript{26} used family-based methods to test for associations between *IL13* SNPs and asthma severity, morbidity, or both in 2 well-characterized, ethnically and geographically distinct groups of asthmatics: Costa Rican children and white non-Hispanic children in the Childhood Asthma Management Program.

*IL13* + 2044GA (rs20541), a coding SNP that leads to the synthesis of an IL-13 variant with enhanced biologic activity,\textsuperscript{27} was significantly associated with an increase in eosinophil counts and serum total IgE levels in both populations. Interestingly, *IL13* − 1112CT (rs1800925), which results in increased *IL13* transcription and expression in TH2 cells,\textsuperscript{28} appeared to be inversely associated with asthma exacerbations in Costa Rica but was associated with increased risk of asthma exacerbations among children receiving inhaled corticosteroids. These results highlight a potentially novel aspect of *IL13* biology: an effect on asthma exacerbations and response to treatment. That *IL13* − 1112CT (rs1800925), a gain-of-function SNP, protects against exacerbations is puzzling but might reflect the involvement of different cell types in basal allergic inflammation and asthma exacerbations.\textsuperscript{29}

Interesting insights into the role of *IL13* in airway disease were also provided by Ermers et al,\textsuperscript{30} who studied the clinical, immunologic, and genetic determinants of persistent or late wheezing after a lower respiratory tract infection (LRTI) caused by respiratory syncytial virus (RSV). Following a cohort of 101 children hospitalized for an RSV-induced LRTI prospectively for 6 years, these authors found that atopic family history was associated with late, but not early, wheezing. Most importantly, the minor A allele at *IL13* + 2044 (rs20541) was also strongly associated with late, but not early, wheezing, raising the possibility that early and late wheezing after RSV LRTI might be caused by distinct pathophysiologic mechanisms. The effect of *IL13* + 2044GA (rs20541) on IL-13 function was further supported by another study showing increased activity of the IL-13 R130Q protein variant on cells expressing low IL-13 receptor α2 levels.\textsuperscript{31}

Replication of results across studies remains the gold standard to assess the robustness of genetic associations. However, virtually no genetic association has been universally replicated, especially using strict criteria (same polymorphism, same phenotype, and same direction of the effect).\textsuperscript{32} This is why meta-analyses that reassess published association data according to well-defined, strict criteria are useful to define the extent to which variants in a given gene truly modify disease susceptibility. One such meta-analysis was recently performed by Loza and Chang\textsuperscript{32} for *IL-4R*, which encodes the α chain of the IL-4 and IL-13 receptors. After reviewing multiple case-control association studies that met specified inclusion criteria (9 and 8 studies for *IL4R*500V and Q551R, respectively), the authors concluded that *IL4R*551, but not *IL4R*500V, is significantly associated with increased risk of asthma, most notably atopic asthma. Interestingly, according to Baynam et al,\textsuperscript{33} the effects of *IL4R* appear to be context dependent: in a study of infant vaccine responses, the *IL4R* 551QR/QQ genotypes were associated with significant decreases in IgG levels and T-cell responses (IFN-γ, IL-10, and IL-13) to tetanus toxoid and parallel reductions in polyclonal T-cell responses and innate immune responses but only in tobacco smoke–exposed infants, pointing to a gene-environment interaction between an *IL4R* variant and passive smoke exposure in early life.
FLG, A NOVEL CANDIDATE GENE FOR ATOPIC DERMATITIS

Null mutations in FLG, a member of the epidermal differentiation complex on chromosome 1q21, were recently reported to be strongly associated with atopic dermatitis and eczema.\(^{34,35}\) According to the meta-analysis performed by Baurecht et al,\(^{36}\) the effect of FLG variants on the risk of atopic dermatitis exceeds that of any other candidate gene investigated thus far and makes FLG one of the strongest genes known to date for complex diseases. Yet the FLG mutations that predispose to atopic dermatitis are extremely rare. Indeed, their association with the disease was tested only because these mutations are known to cause ichthyosis vulgaris, a common recessive Mendelian disorder of skin keratinization, and atopic dermatitis was highly prevalent among patients with ichthyosis vulgaris who were null or heterozygous for FLG.\(^{37,38}\) These findings underscore both the critical role of an intact epithelial barrier in protecting against environmental agents\(^{38}\) and the complexities of candidate gene discovery.

Mutations in FLG were initially identified in European families. Nomura et al\(^{39}\) studied the role of FLG mutations in ichthyosis vulgaris and atopic dermatitis in Japan. Interestingly, the R501X and 2282del4 mutations originally identified in Europeans were absent in the Japanese population, but 2 novel mutations (3321delA and S2554X) were identified by means of resequencing. Both mutations led to a striking reduction of keratin 5 and 14 granules in the epidermis and were significantly associated with atopic dermatitis. Thus FLG mutations in Japan are distinct from those found in European populations but have a comparable effect on disease susceptibility.

The ability of FLG mutations to influence asthma susceptibility directly or through an effect on atopic dermatitis is still controversial. Palmer et al\(^{40}\) showed that FLG mutations are associated not only with eczema-associated asthma susceptibility but also with asthma severity independent of eczema status. In contrast, Rogers et al\(^{41}\) concluded that FLG loss-of-function mutations do not appear to influence either susceptibility to asthma or asthma severity phenotypes. These discrepancies are likely due to the current lack of populations in which the atopic dermatitis and asthma phenotypes exist independently in groups large enough to allow for a robust statistical analysis. Targeted prospective studies might therefore be necessary to resolve this issue. Of note, FLG is expressed in the epidermis and in the oral and nasal mucosa, although not in the bronchial mucosa.\(^{42}\) If true associations between asthma and FLG variants were to be found only in patients with atopic dermatitis, asthma in individuals with atopic dermatitis might then be secondary to allergic sensitization that occurs after the breakdown of the epidermal skin barrier.\(^{43}\)

REFERENCES


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