

Editorials

Genetic association studies: What are they teaching clinicians?

Airway smooth muscle tone is regulated by parasympathetic fibers carried within the vagus nerve. Of the 5 muscarinic receptors identified to date, 3 have been isolated in human lungs.¹ In asthma acetylcholine released after stimulation of cholinergic nerves binds to M3 muscarinic receptors located in airway smooth muscle and submucosal glands, leading to smooth muscle contraction and bronchoconstriction.² This response can be reproduced clinically with methacholine, an acetylcholine analogue.² In this issue Donfack et al³ hypothesized that polymorphisms in the gene encoding cholinergic receptor 3 (*CHRM3*) might be associated with airway hyperresponsiveness to cholinergic stimuli, leading to asthma, atopy, or both. Fenech et al⁴ previously investigated the coding region of this gene by screening 184 chromosomes but found no polymorphisms that would account for variable responses to cholinergic agonists. This previous study used random asthmatic and control subjects and did not specifically investigate the promoter region, which is the genetic locus where most significant genetic polymorphisms are found. The present study expands on the data presented by Fenech et al by screening the entire 1.2-kb promoter region of the *CHRM3* gene in a well-characterized, highly inbred population. Although the results of this study confirm the findings of other investigators that the *CHRM3* gene is most likely not an asthma susceptibility locus,^{4,5} they did find a significant association with 2 short tandem repeat polymorphisms and skin prick test sensitization to cockroach allergen. The authors speculate that the unexpected association of *CHRM3* with atopy is similar to the association previously reported between the β_2 -adrenergic receptor (*ADRB2* gene), total IgE (glu27 allele), and specific IgE (arg16 allele) levels.^{6,7} These associations are plausible given that both the *ADRB2* and *CHRM3* genes are expressed on T cells, which regulate multiple pathways in asthma.⁸

The cloning of the entire human genome along with microarray technology is sure to result in an exponential increase in the number of genetic association studies in the near future. Numerous studies have already reported associations between a variety of genetic single nucleotide or haploid polymorphisms and asthma-allergic phenotypes. For example, a strong association has been identified between the *R576IL4RA* allele, atopy, and asthma severity.^{9,10} Allelic variation at the glutathione-S-transferase locus has been associated also with atopy and asthma.¹¹ Other studies include associations of β_2 -adrenergic receptor and CD14 (endotoxin receptor) polymorphisms and asthma phenotypes, allergic phenotypes, or both.^{12,13}

In anticipation of the steady stream of genetic association studies expected in the near future, it is essential that the clinician be able to interpret these findings in their proper clinical context. First and foremost, it is important to understand that these are association studies and not functional studies. In other words most of these studies have not demonstrated that the specific genetic polymorphism in question is functionally responsible for a specific asthma-allergic phenotype or their characteristic pathophysiologic features. Future functional studies, which are much more complex to design and execute, are the logical extension of these association studies. Therefore association studies primarily provide new hypothetical approaches for developing future translational and clinical research investigations.

The features that constitute a well-designed genetic association study are of paramount importance to the clinician. Donfack et al³ incorporate many of the requisites recommended for conducting genetic association studies in asthma. First, these investigators used a highly inbred Hutterite population, thereby ensuring ethnic homogeneity and eliminating the potential problem of population stratification.^{3,14,15} Evaluation of diverse ethnic populations might lead to erroneous associations of allele frequencies in both patients and control subjects. Second, genetic assessment of a single pedigree in which subjects are all related increases the statistical power and the likelihood that associations are reliable.¹⁵ Several nontraditional epidemiologic approaches for analysis of gene-environment interaction have been evaluated that do not require a control population.¹⁶ For example, in case-only studies, an association between exposure and a specific genotype can be examined among patients without the use of a control population. Odds ratios are interpreted as a synergy index on a multiplicative scale on the basis of the assumption that there is no relationship between the genotype and exposure.¹⁶ In case-parental

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control studies the genotypic distribution of patients can be compared with the expected distribution on the basis of parental genotypes in which no association between genotype and disease is present. These designs have been demonstrated to permit smaller sample sizes to achieve statistical significance compared with more traditional case-control and case-sibling designs.¹⁷ Application of the statistical methods used for case-only studies might help to further explain the unexpected association between cockroach sensitization and nonrandom transmission of haplotypes in the relatively small population of Donfack et al.³ Finally, they appropriately applied the transmission-disequilibrium test to assess for nonrandom transmission of alleles or haplotypes in affected individuals of the Hutterite population. Linkage disequilibrium occurs because alleles of closely linked genetic loci segregate together with increased frequency across a population.¹⁵ Linkage disequilibrium most likely further accounts for the association of the 3 short tandem repeat polymorphisms identified in their population with cockroach sensitization, a presumed marker of atopy. A shortcoming of this study, which is a common problem of most association studies, was incomplete characterization of their small control population with respect to asthma and atopic status.¹⁵

Although the results reported by Donfack et al³ do not yet appear to be widely applicable, they should not be overlooked as being entirely irrelevant. Rather, they represent an important additional piece of the puzzle that might eventually unravel the complexity of the human asthma genotype. The clinical allergist's charge in the 21st century should be to critically determine whether such published association studies are providing relevant information that advances our understanding of asthma. Furthermore, allergists should become better phenotypers by accurately establishing atopic status and disease severity status of their patients, so that when genotyping becomes a routine clinical reality, they will be prepared to use this unique tool for appropriate diagnostic and therapeutic interventions.

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Correction

In the NAEPP Report, "National Asthma Education and Prevention Program Expert Panel Report: Guidelines for the Diagnosis and Management of Asthma/Update on Selected Topics—2002," published as a supplement to *The Journal of Allergy and Clinical Immunology* in November 2002 (*J Allergy Clin Immunol* 2002;110[5 Suppl]:S141-S210), there were errors in Table 1-2c and Appendix A-2. The corrections are as follows.

Table 1-2c: In the column *Study Arm*, the abbreviation "BDP" is incorrect; it should be "BUD."

Appendix A-2, Figure 3: For the medication *Albuterol, Nebulizer solution*, in the column *Dosage Form*, the second dosage is incorrect. Rather than "2.5 mg/mL," as printed, it should be "2.5 mg/3 mL."