

Predictive Genetic Profiles for β -Agonist Therapy in Asthma

A Future under Construction

The goal of pharmacogenetic research is to determine the effects of genetic variation on the response of a phenotype to a pharmacologic agent. Pharmacogenetics has the potential to identify genetic biomarkers that can be used to stratify individuals for personalized therapies and, thus, place the individual at the center of therapeutic management decisions for precision medicine. Personalized medicine has a potential role in the treatment of asthma as a result of high interindividual variability in therapeutic responsiveness to the different classes of therapy for asthma. Among airways diseases, genetic studies have been successful in the development of personalized therapies for cystic fibrosis in individuals with specific rare coding variants in *CFTR*, yet no genetic variant has been successfully translated to the treatment of asthma (1).

Pharmacogenetic studies addressing inhaled β_2 -adrenergic receptor agonist (β -agonist) response in asthma are unique among the different classes of pharmacologic therapies used to treat asthma, in that these studies address a controversy dating back to the 1960s. Epidemiologic studies identified an increased risk for asthma-related death during treatment with two less-selective inhaled short-acting β -agonists (SABAs) during mortality epidemics through the 1960s and 1970s, which resolved after the withdrawal of these drugs (2, 3). More recently, two large surveillance studies, including the Salmeterol Multicenter Asthma Research Trial, have shown a small increase in life-threatening asthma exacerbations and death during treatment with the long-acting β -agonist (LABA) salmeterol (4). Despite clinical trial and meta-analytical evidence demonstrating the efficacy and safety of LABA when used in combination with an inhaled glucocorticoid, these safety concerns resulted in a boxed warning from the US Food and Drug Administration and a mandatory LABA safety study that is currently enrolling 46,800 patients with asthma (5). Identification of subgroups at risk for adverse responses to β -agonists is critical to understanding the mechanisms that underlie LABA safety and the development of biomarkers for personalized therapies.

Genetic studies for β -agonist response in asthma have included biologic candidate genes in the β_2 -adrenergic receptor and nitric oxide biosynthetic pathways. Most studies have focused on the gene encoding the β_2 -adrenergic receptor (*ADRB2*), which has common coding variants, Gly¹⁶Arg and Gln²⁷Glu, with functional effects *in vitro* (6). Gly¹⁶Arg was associated with acute bronchodilation in response to albuterol and long-term response to regular albuterol treatment; however, this locus did not alter responses to LABA therapy in a number of genetic studies, including two genotype-stratified clinical trials (7–10). In a recent study, two rare *ADRB2* variants were associated with hospitalization during LABA therapy, suggesting a role for rare variants in determining adverse responses (11).

High-throughput whole-genome genotyping has recently provided an opportunity for unbiased genome-wide association studies (GWAS), which have discovered novel pharmacogenetic loci. The first GWAS for acute SABA bronchodilation identified a locus in spermatogenesis-associated serine-rich 2-like gene (*SPATS2L*) in non-Hispanic whites from six clinical trial cohorts

and the National Heart, Lung, and Blood Institute Severe Asthma Research Program. MicroRNA knockdown of *SPATS2L* in human airway smooth muscle cells resulted in increased expression of the β_2 -adrenergic receptor (12). Two recent GWAS have identified additional novel loci associated with SABA bronchodilation, one of which used GWAS and admixture mapping to identify rare variants (allele frequency, <0.05) in solute carrier genes (13, 14). Although some of the common variants identified in these GWAS were replicated in independent cohorts, none met the stringent threshold of genome-wide significance.

In this issue of the *Journal*, Israel and colleagues (pp. 530–537) performed a GWAS of the degree of FEV₁ bronchodilation after two or more inhalations of albuterol in 724 non-Hispanic white patients with asthma from the SHARE-Asthma Resource Project, which identified and replicated associations with four loci adjacent to the ankyrin repeat and SOCS (suppressor of cytokine signaling) box-containing 3 gene (*ASB3*) (15). A meta-analysis of the discovery and replication cohorts identified stronger associations for these single nucleotide polymorphisms (SNPs); thus, this GWAS was the first to report associations reaching genome-wide significance for β -agonist response in asthma. Israel and colleagues also identified plausible relationships between *ASB3* and biologic pathways that regulate smooth muscle proliferation and β_2 -adrenergic receptor signaling, using bioinformatics database analyses to provide supporting biologic evidence for *ASB3* as a pharmacogenetic locus.

Are the variants identified by Israel and colleagues ideal genetic biomarkers for stratified, personalized treatment in an individual patient with asthma? The data presented are suggestive; however, more information is needed. The four SNPs identified in this GWAS were located approximately 1 million bases away from *ASB3* and are likely surrogate-tagging SNPs for another causative variant. More detailed genotyping or DNA sequencing of the genomic region encompassing the regulatory and coding regions containing the SNPs identified in this GWAS and *ASB3* will be necessary. In addition, the role of gene variation in *ASB3* in the regulation of smooth muscle proliferation and β_2 -adrenergic receptor-mediated smooth muscle relaxation must be confirmed with *in vitro* functional studies.

Could this novel pharmacogenetic locus for acute SABA bronchodilation alter responses to long-term, regular β -agonist treatment? The Gly¹⁶Arg locus in *ADRB2* demonstrated contradictory effects on lung function response during acute bronchodilation versus long-term regular SABA therapy (7, 10). Furthermore, Gly¹⁶Arg genotype was not associated with clinical responsiveness to regular LABA therapy in multiple pharmacogenetic studies (8, 9). Therefore, the *ASB3* locus should be evaluated in LABA-treated clinical trial cohorts. An important caveat is that although asthma management guidelines recommend that SABA therapy only be used as a rescue therapy, regular SABA treatment is still used in many areas of the world where asthma controller therapies are not readily available. Thus, the *ASB3* locus could identify a subgroup of patients with asthma in whom regular SABA use is effective. It is also important to note that the clinical trial cohorts evaluated in this GWAS were treated

with varying doses of albuterol in the background of other inhaled therapies, which might have obscured additional novel associations. Future GWAS in cohorts treated with escalating doses of albuterol for maximal bronchodilation after a well-defined drug hold could identify additional loci for SABA response.

How do the findings by Israel and colleagues contribute to our knowledge of the genes underlying the risk for rare, life-threatening adverse responses to β -agonists? A recent genetic study demonstrated a rationale for rare variants influencing these uncommon, severe events (11). The common Gly¹⁶Arg variant has been shown to alter albuterol response in Puerto Ricans, not Mexicans, and the frequency of adverse responses to LABA differs between ethnic groups, suggesting that variants from different ancestries influence β -agonist response (4, 10). As outlined in Figure 1, the associated SNPs adjacent to *ASB3* have varying frequencies in different populations, suggesting that *ASB3* variation might not determine β -agonist response in some groups or that different *ASB3* variants could play a role in different populations (16). More detailed genotyping of *ASB3* and pharmacogenetic studies in ethnically diverse cohorts is needed to answer this question.

The overarching aim of pharmacogenetic research is to identify and validate genetic variants predictive for individual responsiveness to a pharmacologic treatment that can be used as biomarkers for genetic profiles for personalized or stratified medicine. Even if *ASB3* were to be validated as a pharmacogenetic locus for SABA and LABA responsiveness in asthma, it is most likely that causative *ASB3* variants would be a small part of a larger set of molecular and genetic biomarker panels to stratify individuals to different treatment strategies. These genetic profiles for personalized medicine are currently being constructed through pharmacogenetic studies that will increasingly take advantage

of technological advances such as next-generation DNA and RNA sequencing to bring us another step closer to the future of personalized medicine in asthma. ■

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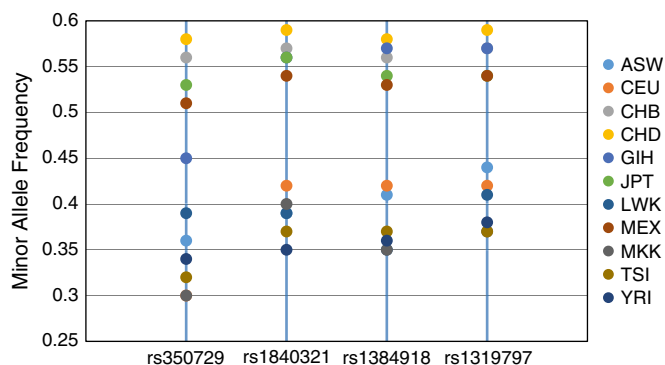


Figure 1. Minor allele frequencies of the top associated *ASB3* loci in different ethnic groups and ancestral populations. These gene loci were identified through a genome-wide association study of β -agonist bronchodilation by Israel and colleagues. Each associated single-nucleotide polymorphism is along the horizontal axis denoted by rs number. Allele frequencies are provided for each ethnic group, and ancestral population along the vertical axes, based on the International HapMap Project Genome Browser release 28, phases 1–3. ASW = African Americans from the southwest United States; CEU = Utah residents with ancestry from northern and western Europe; CHB = Han Chinese from Beijing, China; CHD = Chinese from Denver, Colorado; GIH = Gujarati Indians from Houston, Texas; JPT = Japanese from Tokyo, Japan; LWK = Luhya in Webuye, Kenya; MEX = Mexican Americans from Los Angeles, California; MKK = Maasai in Kinyawa, Kenya; TSI = Tuscan in Italy; YRI = individuals from Yoruba in Ibadan, Nigeria.

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Cysteinyl Leukotrienes: An Innate System for Epithelial Control of Airway Smooth Muscle Proliferation?

Cysteinyl leukotrienes (cysLTs) are products of arachidonic acid metabolism by the 5-lipoxygenase/leukotriene C₄ synthase (5-LO/LTC₄S) synthetic pathway. LTC₄, the parent cysLT, is rapidly synthesized in response to cellular activation, released by a specific transporter, and converted extracellularly to LTD₄ and LTE₄ through the sequential actions of extracellular enzymes (1). CysLTs are generated by activated mast cells, eosinophils, basophils, macrophages, and myeloid dendritic cells, all of which constitutively express the requisite enzymes necessary for the formation of LTC₄. All are hematopoietic cellular constituents of allergic inflammation. CysLTs signal through at least three cognate receptors, termed the type 1 and type 2 cysLT receptors (CysLT₁R and CysLT₂R) (2, 3), and GPR99 (4). Because of their potent effects as smooth muscle spasmogens (5) (an effect attributed primarily to CysLT₁R), and because of evidence that the production of cysLTs increased during spontaneous asthma exacerbations (6), cysLTs drew early interest as potential mediators of asthma. This led to the development of some of the first molecularly targeted drugs for asthma therapy, the 5-lipoxygenase inhibitor, zileuton, and selective CysLT₁R antagonists. Both classes of drugs show efficacy in improving baseline airflow measurements and reducing the frequency of asthma exacerbations (7, 8), supporting the pathobiologic role of the cysLTs in regulating bronchomotor tone in asthma.

Advances in molecular biology have significantly expanded our understanding of the potential pathobiologic functions of cysLTs. In mouse models of allergic pulmonary inflammation, cysLTs play a critical role in the induction and amplification of Th2-type immune responses. Mice lacking LTC₄S or CysLT₁R, with impaired cysLT generation or signaling, respectively, showed a marked reduction in the development of eosinophilic pulmonary inflammation and Th2 immunity in response to intranasal extracts from the house dust mites *Dermatophagoides farinae* or *Dermatophagoides pteronyssinus* (Dp) (9–11). CysLT generation in these studies conditioned dendritic cells for Th2 priming via an autocrine/paracrine action at CysLT₁R. In another model, intranasal delivery of LTC₄ to sensitized mice receiving ovalbumin challenges markedly amplified pulmonary eosinophil recruitment by a pathway involving the activation of CysLT₂R on platelets (12). In a model of intranasal sensitization and challenge to the mold *Alternaria alternata*, the recruitment and activation of type 2 innate lymphoid cells depended on cysLTs (13). Last, in a model of chronic airway inflammation induced by repetitive ovalbumin challenges of sensitized mice, the administration of the CysLT₁R

antagonist montelukast inhibited peribronchial collagen deposition and airway smooth muscle hyperplasia (14). Although the latter finding supports a potential direct role for cysLTs (and CysLT₁R) in driving airway remodeling, the pleiotropic actions of cysLTs at multiple steps in the proximal innate immune response require that downstream effects on structural cells be interpreted with caution.

In this issue of the *Journal*, Trian and colleagues (pp. 538–546) demonstrate a potential pathway by which cysLTs may elicit airway smooth muscle proliferation independent of the proximal immune response (15). They demonstrate that airway smooth muscle cells (ASM) cultured from bronchial biopsies of patients with severe asthma proliferated in response to cysLTs generated by Dp-stimulated airway epithelial cells. ASM from nonasthmatic controls did not respond to the epithelial supernatants and expressed substantially less CysLT₁R mRNA and protein than did cells from severe asthmatics. The effect of Dp on epithelial cells was inhibited by antibodies against protease-activated receptor 2, suggesting Dp proteases were responsible for the activation of the 5-LO/LTC₄S pathway in this system. Treatment of the epithelial cells with either zileuton or dexamethasone eliminated Dp induction of the growth factor activity and LTC₄ synthesis. The epithelial cells showed substantial up-regulation of 5-LO, 5-LO-activating protein, and LTC₄S mRNA transcript expression in response to Dp, suggesting epithelial cells (in contrast to hematopoietic cells) may require transcriptional induction to become competent to generate cysLTs at a level sufficient to cause physiologic effects on smooth muscle.

The study by Trian and colleagues carries several potential implications and caveats. First, although hematopoietic cells almost certainly provide the majority of cysLTs under virtually all circumstances, the study suggests epithelial cells can inducibly express the 5-LO/LTC₄S system and could contribute cysLT synthesis when perturbed by environmental danger signals such as proteases. This would be consistent with previous observations that bronchial epithelial cells up-regulate 5-LO, 5-LO-activating protein, and LTC₄S expression in response to stimulation with bradykinin or lipopolysaccharide (16). Second, epithelially derived cysLTs elicited proliferation only in asthmatic ASM, but not control ASM, which was attributed to the markedly higher levels of CysLT₁R expression by asthmatic ASM. This phenomenon has not been reported previously and seems at odds with the previous reports that patients with asthma and nonasthmatic controls exhibit equivalent sensitivity to bronchoconstriction elicited by inhalation challenges with LTD₄, the preferred ligand for CysLT₁R (17, 18). Because the ASM cells used in

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