Artigo original

The Pharmacogenetics of Asthma and the Road to Personalized Medicine

A Farmacogenética da Asma e o Caminho para a Medicina Personalizada

Victor E. Ortega, Eugene R. Bleecker[1]

RESUMO

A asma é uma doença frequente e heterogênea, tanto em termos de expressão fenotípica, como de resposta aos diferentes tratamentos medicamentosos.

Até o momento, os estudos farmacogenéticos investigaram o papel da variação genética na resposta farmacológica em três classes principais de medicamentos: agonistas dos receptores β_2-adrenérgicos (β_2-agonistas), corticosteroides e modificadores de leucotrienos. Essas análises contribuíram para a compreensão dos determinantes da resposta clínica às diferentes terapias contra asma; porém, a maioria dessas análises é limitada, pois são análises retrospectivas de pequenos grupos populacionais, feitas com base numa abordagem de identificação do gene candidato sujeita a vieses, o que pode requerer replicação em coortes maiores. Estudos farmacogenéticos também vêm investigando determinantes genéticos da resposta a terapias biológicas, tais como a inibição de citocinas por anticorpo.

Abordagens futuras deveriam utilizar ensaios clínicos com abordagens sem vieses, com genomas amplos em grandes populações. Na investigação de eventos incomuns, o ressequenciamento de genes candidatos ou de todo o genoma deveria ser usado para identificar variações genéticas raras com potencial na identificação de efeitos genéticos raros em fenótipos baseados na resposta ao tratamento. Algumas das variantes genéticas que determinam a resposta ao fármaco têm frequência baixa, embora não raras, e deveriam ser validadas através de estudos prospectivos com desenho estratificado por genótipo.

Descritores: Asma/terapia; Asma/genética; Farmacogenética.

ABSTRACT

Asthma is a common disease that is a heterogeneous disorder both in terms of phenotypic expression and its response to different drug therapies.

Asthma pharmacogenetic studies to date have investigated the role of genetic variation in drug response for three major drug classes: the β_2-adrenergic receptor agonists (beta agonists), corticosteroids, and leukotriene modifiers. These analyses have contributed to our understanding of the determinants of clinical response to different asthma therapies but are limited in that, for the most part, they are retrospective analyses of smaller clinical trial populations using what might be a more biased candidate gene approach that requires replication in larger cohorts. Pharmacogenetic studies have also investigated genetic determinants of drug response to biologic therapies such as antibody inhibition of cytokines.

Future approaches should utilize unbiased, genome-wide approaches in larger clinical trial populations. In the investigation of uncommon events, resequencing of candidate genes or whole genome sequencing should be used to identify rare gene variations with the potential to identify rarer genetic effects on drug response phenotypes. Some of the genetic variants that determine drug response have lower frequencies but are not rare and should be validated through prospective studies with a genotype-stratified design.

Keywords: Asthma/therapy; Asthma/genetics; Pharmacogenetics.

1. Wake Forest School of Medicine, Winston-Salem, North Carolina.
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Correspondence to: Dr. Eugene R. Bleecker, M.D. Wake Forest School of Medicine. Medical Center Blvd, Winston-Salem, NC 27157. Tel: 1 (336) 716-2011. E-mail: ebleeck@wakehealth.edu.

INTRODUCTION

Asthma is a common disease affecting more than 300 million people worldwide (1). It is a disease characterized by variable degrees of airflow obstruction and inflammation of the airways resulting from multiple, complex pathways. Asthma is a chronic and complex disease with marked heterogeneity in disease expression determined by the interaction of genetic and environmental factors (2,3). Individuals with asthma are treated with a combination of different short-term, rescue, and long-term, controller medications which include β_2-adrenergic receptor agonists (beta agonists), leukotriene modifiers, inhaled corticosteroids (ICS), systemic corticosteroids, anticholinergics, and theophylline. In the future, biologic therapies will be used in responder subsets that can be identified using pharmacogenetic and biomarker-based approaches.

Asthma is as heterogeneous in its response to different drug therapies as it is in phenotypic expression. An analysis of responses to common asthma therapies demonstrates that 70-80% of patients with asthma exhibit variable clinical responses to these medications. This large variance in drug response is beyond what would be expected by patient adherence alone and suggests that a heritable or genetic factor is involved in determining drug response among asthma patients (4). Despite large differences in drug response between individuals in the general population, intraindividual variability remains low consistent with the role of a heritable factor to drug responses (5,6). In fact, genetic variation might account for a larger percentage of the observed variability in drug response, whether beneficial or adverse (5).

Pharmacogenetics is the study of the role of genetic variability in determining interindividual responses to pharmacological therapies and represents the analysis of a gene by environment interaction where the environment is exposure to a medication (Figure 1). Pharmacogenetic research attempts to characterize genetic determinants and their effects on drug response in two fundamental ways: the analysis of genetic effects on clinical response to a drug resulting in measurable changes in a clinical phenotype (pharmacodynamics) and genetic effects on drug metabolism resulting in toxic or subtherapeutic levels within a target organ (pharmacokinetics).

The majority of pharmacogenetic studies in asthma have been limited to pharmacodynamic endpoints due to the retrospective study design used in the majority of studies in current literature. In a retrospective pharmacogenetic study design, pharmacodynamic or clinical endpoints such as airflow obstruction—as measured by FEV_1 and PEF rate (PEFR)—and asthma exacerbations are analyzed for genotypic associations using DNA from participants in a clinical trial. A small number of pharmacogenetic trials have employed a prospective trial design in which patients with asthma are allocated to treatment or placebo groups based on genotypes from DNA obtained prior to randomization. Retrospective pharmacogenetic analyses are essential for the identification of genetic variants or candidate genes of interest and can employ unbiased genome-wide approaches, whereas prospective, genotype-stratified approaches are appropriately powered for the analysis of genetic variants that might be somewhat less common by permitting the study of an adequate sample size containing the risk genotype.

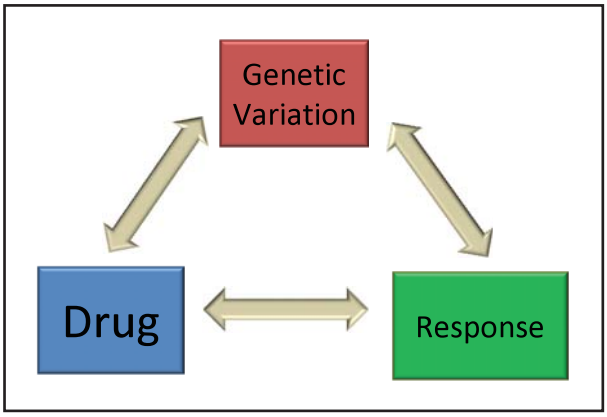

Figure 1 - Pharmacogenetics is the study of the role of genetic variability in determining interindividual responses to pharmacological therapies and represents the analysis of a gene by environmental interaction in which the environment is exposure to a medication.

RELEVANCE OF PHARMACOGENETIC STUDIES IN ASTHMA

Pharmacogenetic research in asthma is driven by two unresolved problems in asthma treatment that would benefit from a personalized approach. The first problem is that a small subset of individuals with asthma (5-10%) experience uncontrolled symptoms and recurrent exacerbations despite treatment with multiple asthma therapies or high doses of ICS (7,9). This subset of severe cases represents a small proportion of the total asthma population; however, this refractory asthma population experiences substantial morbidity and represents a financial burden at least six times greater than that of the population of individuals with milder asthma (9). The second issue is that there are adverse effects related to the use of some asthma therapies, particularly the rare adverse events attributed to medications such as beta agonists (10-12). Overall, the use of pharmacogenetic approaches has the potential of improving personalized therapeutic approaches in which asthma therapies are stratified to optimize therapeutic responses and reduce adverse side effects in an individual.

To date, pharmacogenetic studies of asthma have investigated the role of genetic variability in drug response for three major drug classes including the leukotriene modifiers, corticosteroids, and beta agonists. We will also discuss how pharmacogenetic approaches have recently allowed investigators to identify a subset

of patients that might benefit from these agents. We will then conclude with present and future approaches that have the potential to bring us closer to an era of personalized medicine.

PHARMACOGENETICS OF THE LEUKOTRIENE PATHWAY

The cysteinyl leukotriene pathway plays an important role in the pathogenesis and treatment of asthma in a subset of patients. Cysteinyl leukotrienes mediate a variety of biological processes relevant to asthma, including smooth muscle contraction and allergic airways inflammation through eosinophil migration. Leukotrienes are synthesized by a cascade of enzymes initiated by the conversion of arachidonic acid to leukotriene A_4 by 5-lipoxygenase (5-LO), the rate-limiting step of this pathway encoded by the *ALOX5* gene. Subsequent steps in the leukotriene biosynthetic cascade include enzymes that mediate the conversion of leukotriene A_4 to leukotriene B_4 (leukotriene A_4 hydrolase encoded by *LTA4H*), leukotriene C_4 (leukotriene C_4 synthase encoded by *LTC4S*), or leukotriene D_4. Leukotriene C_4 is transported to the extracellular space by the multi-drug resistance protein 1, a genetic product of *MRP1*. Leukotriene C_4 and leukotriene D_4 signal biologic effects by binding and activating cysteinyl leukotriene receptors, which are G-protein coupled receptors that are a product of the *CYSLTR1* and *CYSLTR2* genes. As depicted in Figure 2, genetic variations in this pathway have been associated with asthma susceptibility but also are genetic determinants of response to medications that target this pathway (13-17).

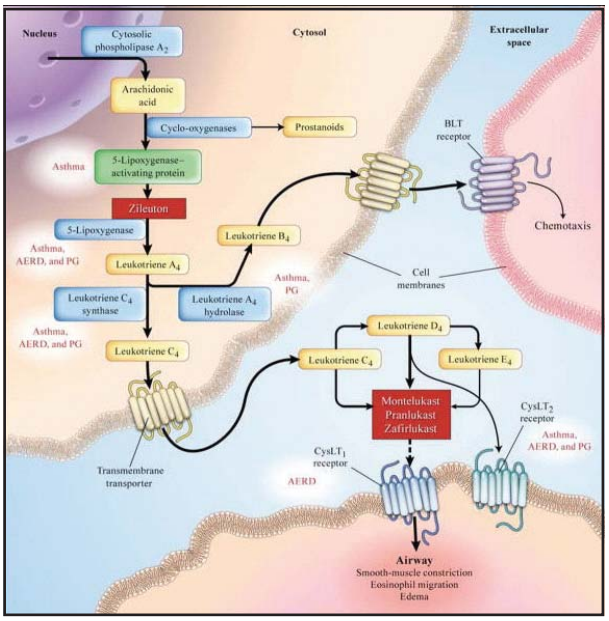

Figure 2 - Overview of the leukotriene pathway. Boxes highlighted in red indicate known genetic associations.

AERD: aspirin exacerbated respiratory disease; PG = pharmacogenetic.
Reproduced from Tantisira and Drazen (17).

There are two major classes of medications that target the cysteinyl leukotriene pathway for the management of asthma: 5-LO inhibitors and cysteinyl leukotriene receptor 1 antagonists (pranlukast, montelukast, and zafirlukast), as well as other leukotriene modifiers under current development. When asthma study populations are analyzed, these agents are associated with beneficial effects on lung function and symptom control (18,19). Despite these effects, a crossover trial randomizing 126 children with asthma to either an ICS (fluticasone) or a leukotriene receptor antagonist (montelukast) demonstrates inter-individual variability in the response to these agents. Treatment with fluticasone or montelukast resulted in improvements in lung function as measured by FEV_1 in asthma (Figure 3); however, 17% had a treatment response (i.e., ≥ 7.5% increase in FEV_1) to both medications, 23% responded to the corticosteroid alone, 5% responded only to montelukast, and 55% did not show a response to either medication; certain subsets also experienced adverse responses to one or both medications (20). This interindividual variability in the response to montelukast and ICS therapy emphasizes the rationale for pharmacogenetic research to identify therapies that are efficacious, ineffective, or even harmful for individual asthma patients.

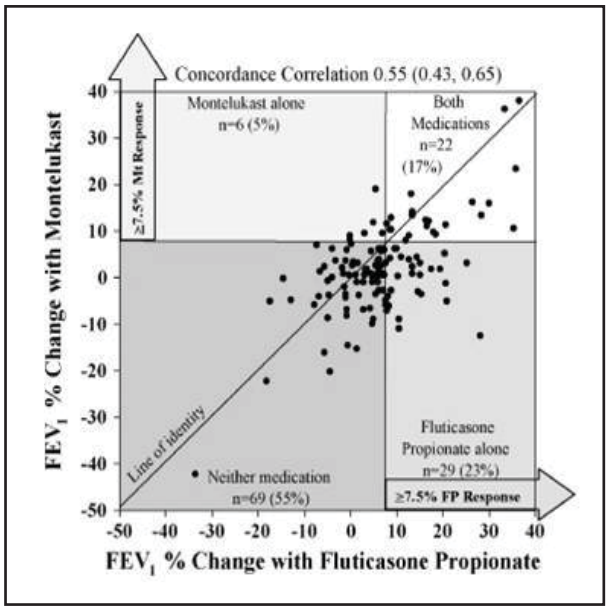

Figure 3 - Variability of response and differential response to fluticasone and montelukast, as measured by change in FEV_1. Four categories of response are displayed, with a favorable response defined as an increase of ≥ 7.5% in FEV_1. The line of identity designates patients favoring montelukast above the line, and those favoring fluticasone below the line.

Reproduced from Szefler et al. (20).

In an early pharmacogenetic analysis in asthma, Drazen et al. performed a retrospective candidate gene analysis of the *ALOX5* gene through a clinical trial consisting of asthma patients treated with a 5-LO inhibitor,

ABT-761 (21). The *ALOX5* gene contains a variable tandem repeat of a transcription factor binding motif that had previously been shown to reduce gene transcription *in vitro*; thereby, potentially diminishing 5-LO activity and reducing downstream cysteinyl leukotriene synthesis. In that clinical trial cohort, 104 homozygotes and heterozygotes for the common allele of the *ALOX5* promoter experienced significant improvements in FEV_1 (18.8-23.3%) with ABT-761, respectively. In contrast, participants who were homozygotes for the variant *ALOX5* promoter region did not respond to the 5-LO inhibitor (−1.2% change in FEV_1). These findings were replicated in a smaller clinical trial of 61 patients treated with montelukast, further demonstrating that asthma patients with the *ALOX5* promoter region variant have a reduced therapeutic response (22).

Pharmacogenetic studies of the leukotriene pathway in patients with asthma have also included retrospective candidate gene analyses of other pathway-related genes. Lima et al. genotyped 28 single-nucleotide polymorphisms (SNPs) in genes throughout the pathway using DNA from 61 non-Hispanic White participants with poorly controlled, mild to moderate persistent asthma randomized to treatment with montelukast. The SNPs in *ALOX5* (rs2115819) and *MRP1* (rs119774) were found to be significantly associated with a change in FEV_1 in response to montelukast (Figure 4). In addition, the variant or minor allele of a promoter SNP in *LTC4S* (rs730012) was associated with a reduced risk of exacerbation while the minor variant of an intronic SNP in *LTA4H* (rs2660845) was associated with an increased risk of exacerbation during montelukast therapy (23).

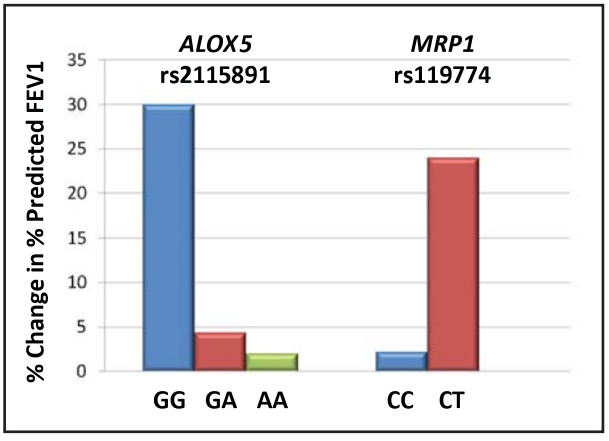

Figure 4 - Influence of genotype on percentage change in % of predicted FEV_1. Percentage increase in FEV_1 (% of predicted) over baseline after 6 months of montelukast treatment in patients with the *MRP1* rs119774 and *ALOX5* rs2115819 genotypes.

Adapted from Lima et al. (23).

These pharmacogenetic studies demonstrate that genetic variation in the leukotriene pathway contributes to the variability in drug response with medications that target this pathway. Larger clinical trials with retrospective candidate gene analyses of the leukotriene pathway or prospective trials should be performed to replicate these findings in order to determine how variation in this pathway can be used in guiding therapy.

PHARMACOGENETICS OF THE CORTICOSTEROID PATHWAY AND PERSONALIZING ASTHMA THERAPY

Corticosteroids or glucocorticoids are the primary anti-inflammatory medication used in the management of asthma. Inhaled glucocorticoids have consistently been shown to have a greater effect on lung function and asthma symptom control when compared with the leukotriene receptor antagonists. Despite this observation, there is a subset of patients who are less responsive to glucocorticoids and might respond to other asthma controller medications (19,20). In asthma subjects who are less responsive to ICS (Figure 3), pharmacogenetic approaches might lead to improved personalized approaches to controller therapy with ICS in asthma (3,8). For example, high-dose ICS might not be preferable in a less responsive asthma patient and the use of an alternative controller therapy may be considered.

The pharmacogenetics of the corticosteroid pathway is based on multiple potential candidate genes encompassing the biosynthesis of glucocorticoids, the cytosolic glucocorticoid receptor heterocomplex, and the chaperone proteins that bind glucocorticoid receptors during the resting state within the cytosol. Glucocorticoids exert their anti-inflammatory effects by activating receptor-chaperone complexes that translocate in the nucleus to repress the transcription factors of pro-inflammatory genes and bind to glucocorticoid response elements in the promoter of anti-inflammatory genes (24,25). Pharmacogenetic studies have investigated the role of candidate genetic variation within the corticosteroid pathway and its impact on the response to corticosteroid therapy.

One of the earliest pharmacogenetic studies investigating glucocorticoid response involved the glucocorticoid receptor gene (*NR3C1*) located in chromosome 5q31, a chromosomal region associated with asthma and related phenotypes in family-based linkage studies (26-28). In a cohort of 216 elderly participants, a nonsynonymous SNP at codon 363 resulted in an asparagine-to-serine substitution. The resulting serine, $Asn^{363}Ser$, was assessed for genotypic effects on glucocorticoid response. Of those 216 participants, 13 (6%) had the variant allele and showed greater sensitivity to glucocorticoids, as determined by cortisol and insulin responses to dexamethasone suppression testing (29).

Genetic variation within the corticosteroid biosynthetic pathway has the potential to determine endogenous glucocorticoid levels and influence the therapeutic response to corticosteroid therapy. A retrospective pharmacogenetic analysis performed by Tantisira et al. investigated SNPs in the corticotropin-releasing

hormone gene (*CRHR1*) in three different clinical trial populations where participants were randomized to ICS therapy: a primary population of 470 adult asthma patients (hereafter, "Adult Study"), 311 childhood asthma patients from the Childhood Asthma Management Program (CAMP), and 336 adult asthma patients from the Asthma Clinical Research Network (ACRN) of the United States National Heart, Lung and Blood Institute. A *CRHR1* SNP, rs242941, was associated with variation in the lung function response to ICS. In the "Adult Study" and CAMP populations (Figure 5), there was a doubling of the FEV_1 response among T homozygotes (TT genotype) when compared with G homozygotes (GG genotype). Another *CRHR1* SNP, rs1876828, was also associated with corticosteroid response in the ACRN population, in which there was a greater FEV1 response among asthma patients with the AA genotype than among those with the GG genotype (30).

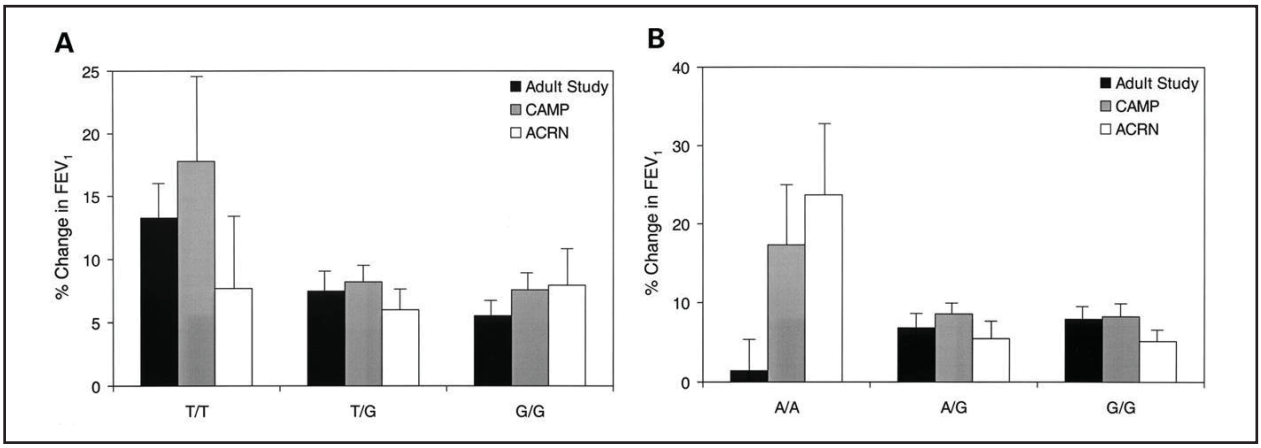

Figure 5 - *CRHR1* genotypes and association with longitudinal response to ICS in asthma patients, adjusted for age, sex, height, and baseline FEV_1. The SNP rs242941 was associated with response over 8 weeks in the "Adult Study" and CAMP populations. The SNP rs1876828 was associated with response over 6 weeks in the ACRN population.

Reproduced from Tantisira et al. (30).

Multiple genes encode for the heterocomplex of chaperones and immunophilins that bind the glucocorticoid receptor and mediate proper assembly and activation of the receptor. Hawkins et al. performed a retrospective genetic analysis of genes encoding for the heterocomplex of chaperones in a clinical trial of 450 asthma patients randomized to treatment with ICS (31). The authors found that genetic variations within the gene encoding for the heat shock organizing protein, *STIP1*, were significantly associated with improvement in FEV_1 response after four weeks of corticosteroid therapy (associated with the SNPs rs6591838 and rs2236647), as well as after eight weeks of the same (associated with the SNPs rs6591838 and rs1011219).

A major challenge in the pharmacogenetic investigation of the corticosteroid pathway lies in its interactions with other genes or pathways that are also regulated by glucocorticoids, such as the β_2-adrenergic receptor pathway (32,33). This challenge is best illustrated in the pharmacogenetics of the *TBX21* gene which encodes for the T-box expressed in the T-cell transcription factor, which influences the development of naïve T lymphocytes. A retrospective candidate gene analysis of *TBX21* in the CAMP clinical trial cohort demonstrated that a nonsynonymous SNP, $His^{33}Glu$, determined improvement in bronchial hyperresponsiveness in response to ICS therapy (34). The pleiotropic effects of glucocorticoids make the study of the corticosteroid pathway crucial to understanding the pharmacogenetics of asthma and call for unbiased, genome-wide approaches be employed.

A recent study was based on a small, family-based, genome-wide association study with replication in additional cohorts. The authors of that study demonstrated a novel pharmacogenetic determinant of ICS response in 118 asthma probands from the CAMP cohort randomized to ICS (budesonide) treatment. The investigators analyzed 13 significantly associated SNPs in four independent replication cohorts totaling 935 asthma patients. The analysis identified a SNP in the promoter region of the glucocorticoid-induced transcript 1 gene (*GLCCI1*), rs37972, which was associated with lung function responses to inhaled glucocorticoids among the CAMP probands and the 935 participants from the replication cohorts (Figure 6). The SNP rs37972 is also in strong linkage equilibrium (i.e., strongly correlated, or "tagged") with another *GLCCI1* promoter SNP, rs37973, which determines gene transcription *in vitro*, demonstrating a functional or molecular-based rationale for the observed genetics effects of variation in this gene on corticosteroid response (35). It will be important to replicate these corticosteroid response gene variants in other, larger populations and determine whether they are independent predictors or have additive effects that regulate corticosteroid responses in asthma.

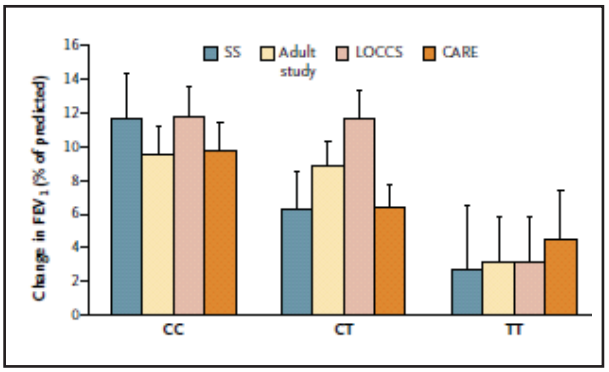

Figure 6 - The association of GLCCI1 rs37972 genotypes (CC, CT, and TT) with change in lung function as a change in FEV_1, expressed as a percentage of the predicted value, after 4 to 8 weeks of therapy with inhaled glucocorticoids in four replication populations: the Salmeterol or Corticosteroids and the Salmeterol with or without Inhaled Corticosteroids trials (SS); the "Adult Study"; the Leukotriene Modifier or Corticosteroid Salmeterol (LOCCS) trial; and the Childhood Asthma Research and Education (CARE) Network trials.

Liptak combined P = 0.0007.

Reproduced from Tantisira et al. (35).

THE B_2-ADRENERGIC RECEPTOR PATHWAY

Inhaled beta agonists are the most commonly prescribed medical therapies for the management of asthma. Inhaled beta agonists exist in two classes: the short-acting beta agonists (SABA: fenoterol, isoproterenol, pirbuterol, levalbuterol, and albuterol) and the long-acting beta agonists (LABA: salmeterol and formoterol). The LABA therapy is administered in combination with an ICS as regular controller therapy, while SABA therapy is used for rescue, as-needed treatment for acute symptom relief or the prevention of exercise-induced symptoms (36). Beta agonists bind to the extracellular β_2-adrenergic receptor, a seven-transmembrane receptor which activates a G-protein coupled receptor pathway through adenylyl cyclase type 9 activation, resulting in airway smooth muscle relaxation (37).

Despite the common use of these agents, this drug class is the center of a controversy related to concerns over adverse events beginning in the 1960s, when high doses of SABAs with less selective β_2 adrenergic receptor activity were associated with serious adverse effects (including death), which resulted in the withdrawal of the SABAs isoproterenol and fenoterol from the market (38-42). Additional data from Sears et al. showed that the regular use of fenoterol results in a loss of asthma symptom control (43). The ACRN Beta Agonist Study (BAGS) demonstrated that regular albuterol was not harmful, albeit no more effective than as-needed therapy for symptom control (44).

Two recent surveillance studies have raised concerns about an increased risk for asthma-related life threatening exacerbation and death among patients with asthma randomized to the addition of LABA to current medical therapy (11,12). These observations and a subsequent meta-analysis based on these findings have resulted in a black-box warning from the United States Food and Drug Administration for all inhalers containing LABAs (10-12). Subsequent randomized, placebo-controlled clinical trials, large meta-analyses, and case-control analyses have not shown an increased risk for life-threatening or fatal adverse events when LABA is administered with an ICS (45-49). In addition, LABA-ICS combination therapy results in improvement in exacerbation rates and symptom control, suggesting that these adverse events are exceedingly rare (45,50-53). The potential for heterogeneity in beta agonist response should be borne in mind, because various pharmacogenetic studies have attempted to identify the small subset of patients with asthma who are susceptible to rare adverse responses to beta agonist therapy.

Initially, pharmacogenetic analyses focused on SABA therapy, LABA therapy, and the encoding of the β_2-adrenergic receptor gene, *ADRβ2* (Figure 7), which is a small intronless gene located in chromosome 5q31, a region linked to asthma and related phenotypes (26-28). The first detailed mutational analysis of *ADRβ2* was performed in 1992 by Reihsaus et al. (54), who characterized nine genetic variants including $Gly^{16}Arg$, $Gln^{27}Glu$, $Val^{34}Met$, and $Thr^{164}Ile$. As can be seen in Figure 7, other investigators have identified 49 polymorphisms spanning the 5' promoter, coding region, and 3' untranslated region of *ADRβ2* (55,56).

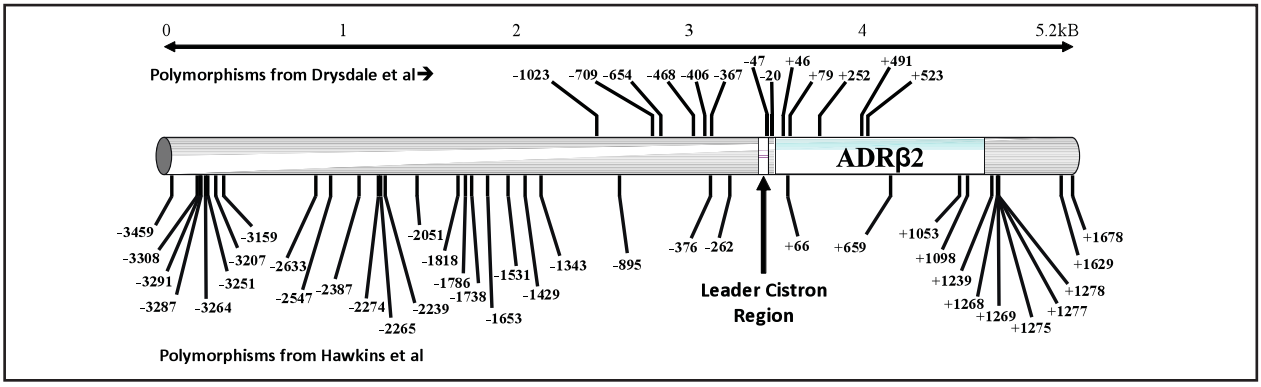

Figure 7 - Diagram of the β_2-adrenergic receptor gene (*ADRβ2*) with polymorphisms denoted by nucleotide position relative to the start codon, in two separate studies.

Adapted from Drysdale et al. and Hawkins et al. (55,56).

In Chinese hamster fibroblasts and human airway smooth muscle cells, Gly^{16}Arg and Gln^{27}Glu have been shown to downregulate the receptor response to beta agonist *in vitro*. The Gly^{16} variant results in enhanced receptor downregulation in response to isoproterenol compared with Arg^{16}, whereas Gln^{27} results in resistance to receptor downregulation compared with Glu^{27} (57,58). These common SNPs have been the focus of multiple candidate gene analyses of *ADRβ2*.

One of the earliest pharmacogenetic studies involving *ADRβ2* was conducted by Martinez et al. (59), who demonstrated that Arg^{16} homozygotes and Gly^{16}Arg heterozygotes were, respectively, 5.3 times and 2.3 times more likely to respond to albuterol than were Gly^{16} homozygotes. This effect was not observed for Gln^{27}Glu (59). Subsequently, Silverman et al. demonstrated that, among the children in the CAMP cohort, the Arg^{16} homozygotes had the highest post-bronchodilator FEV_1 (percentage of predicted) in response to albuterol (60). Other investigators have replicated the genotypic effects of Gly^{16}Arg on the bronchodilator response to a one-time administration of albuterol in smaller populations of asthma patients that have included ethnic groups such as Puerto Ricans (61-64).

Drysdale et al. analyzed estimated *ADRβ2* haplotypes using 13 polymorphisms and reported seemingly contrasting effects of Gly^{16}Arg on bronchodilator responses to a SABA (albuterol). The authors proposed 12 haplotypes, including the Gly^{16}-containing "haplotype 2", which was associated with higher levels of gene transcription and translation when compared with the Arg^{16}-containing "haplotype 4." These *in vitro* findings also corroborated with the *in vivo* finding that haplotype 2 homozygotes experienced the greatest degree of FEV_1 albuterol bronchodilation, while haplotype 4 homozygotes experienced the lowest (55). In a larger resequencing analysis, these haplotype effects were not observed (56).

Pharmacogenetic studies of *ADRβ2* and the response to regular SABA therapy have also focused on variations at Gly^{16}Arg. A retrospective analysis of the ACRN BAGS trial investigated the effects of Gly^{16}Arg and Gln^{27}Glu in 190 participants with mild asthma who were randomized to regular or as-needed albuterol over a 16-week period. The Arg^{16} homozygotes randomized to regular albuterol therapy experienced a decline a PEFR, whereas no such effect was observed among Gly^{16} homozygotes or those randomized to as-needed albuterol therapy, as shown in Figure 8 (65).

Taylor et al. also performed a retrospective candidate gene analysis of a placebo-controlled, cross-over trial consisting of 106 patients with asthma randomized to salmeterol or regularly scheduled albuterol therapy. The Arg^{16} homozygotes experienced a decline in PEFR and a higher frequency of exacerbations during regular albuterol therapy; however, no adverse effects were noted during salmeterol therapy (66). This pharmacogenetic finding has been replicated in retrospective and prospective candidate gene analyses performed by ACRN investigators (65,67). The results of this retrospective genetic analysis and the analysis of the BAGS trial led to the design and implementation of the ACRN Beta Agonist Response by Genotype (BARGE) trial (67).

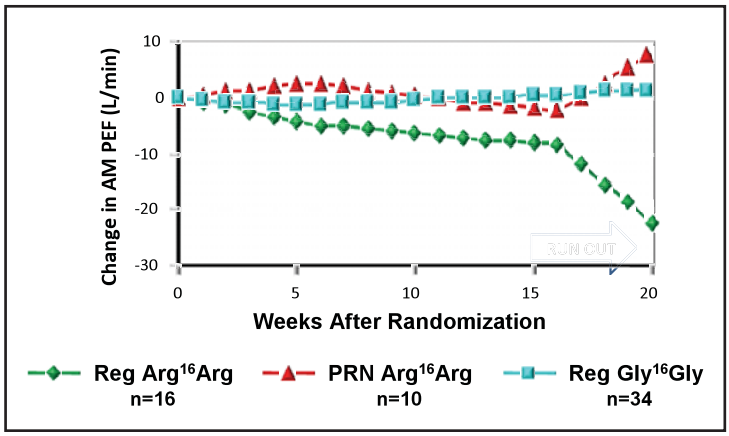

Figure 8 - A retrospective analysis of the BAGS trial investigating the effects of Gly16Arg and Gln27Glu in 190 participants with mild asthma who were randomized to regular or as-needed albuterol over a 16-week period. Arg16 homozygotes randomized to regular albuterol therapy experienced a decline in morning (AM) PEFR, although no such effect was observed among Gly16 homozygotes or those randomized to as-needed albuterol therapy.

Reproduced from Israel et al. (65).

The BARGE trial was one of the first prospective, genotype-stratified, placebo-controlled, cross-over trials where 37 Arg^{16} homozygotes and 41 Gly^{16} homozygotes were randomized to 16-week treatment with regular albuterol or placebo with both groups receiving ipratropium as a rescue inhaler to minimize beta agonist exposure throughout the trial. The Arg^{16} homozygotes experienced no change in PEFR during regular albuterol treatment; however, PEFR improved during intermittent treatment. In contrast, Gly^{16} homozygotes experienced an improvement in PEFR during regular albuterol therapy. The Arg^{16} homozygotes also experienced reduced responses (FEV_1, FVC, asthma symptom scores, and rescue inhaler use) during regular albuterol therapy, whereas Gly^{16} homozygotes experienced improvement in those same endpoints, as can be seen in Figure 9 (67). The contrasting effects of Gly^{16}Arg during acute, one-time exposure versus regular, chronic SABA therapy is thought to be related to variation in receptor kinetics or the pro-inflammatory effects of beta agonists (51,68-70).

Investigators subsequently hypothesized that the observed effects of the Gly^{16}Arg locus on the response to SABA therapy might apply to adverse responses to LABA therapy. Taylor et al.'s The retrospective candidate gene analysis conducted by Taylor et al. did not show genotypic effects at the Gly^{16}Arg locus among

106 asthma patients; however, a small retrospective candidate gene analysis of two ACRN clinical trials demonstrated that, during salmeterol treatment, Arg^{16} homozygotes experienced a significant deterioration in PEFR, asthma symptom scores, and rescue inhaler use when compared with Gly^{16} homozygotes (71). This retrospective pharmacogenetic finding, albeit from two small cohorts, prompted retrospective candidate gene analyses in larger clinical trial populations and two prospective, genotype-stratified trials. Bleecker et al. genotyped five *ADRβ2* SNPs in 183 asthma patients randomized to salmeterol with concomitant ICS therapy or montelukast and demonstrated that all participants experienced sustained and significant improvement in morning PEFR despite the $Gly^{16}Arg$ genotype (72). Subsequently, two cohorts of 2,250 and 405 asthma patients, respectively, randomized to salmeterol or formoterol with concomitant ICS therapy, were genotyped for 11 *ADRβ2* SNPs and did not show significant differences between $Gly^{16}Arg$ genotypes in terms of the time to first exacerbation, PEFR, FEV_1, or rescue inhaler use (73).

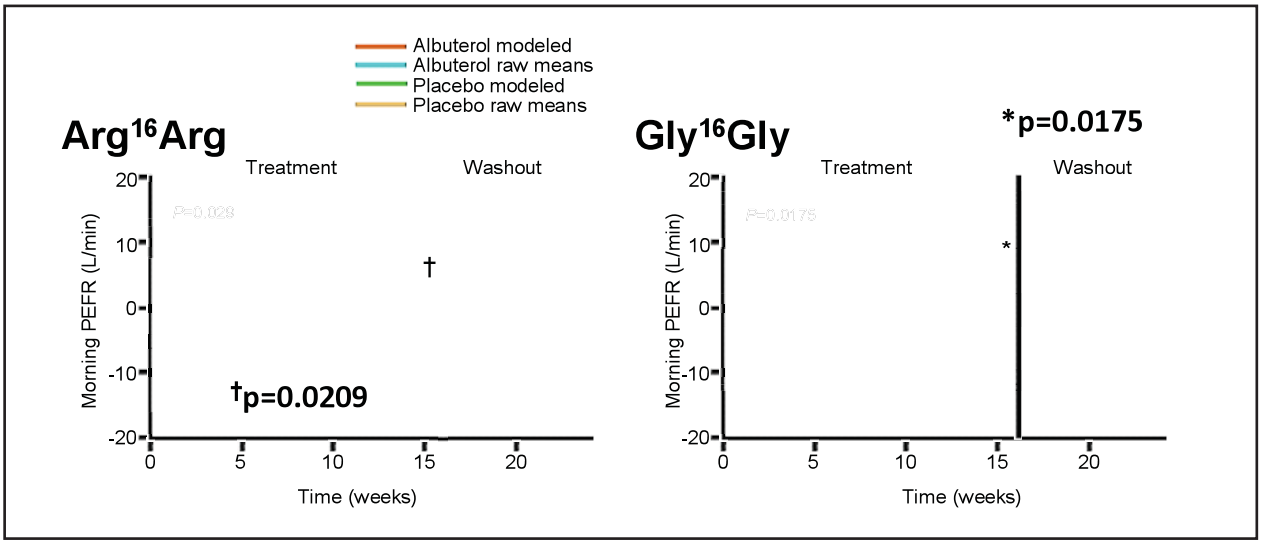

Figure 9 - The BARGE trial was a prospective, genotype-stratified, placebo-controlled, cross-over trial in which 37 Arg16 homozygotes and 41 Gly16 homozygotes were randomized to 16-week treatment with regular albuterol or placebo, both groups receiving ipratropium as a rescue inhaler to minimize beta agonist exposure throughout the trial. Arg16 homozygotes experienced no change in PEFR during albuterol treatment; however, PEFR improved during placebo treatment. Nevertheless, Gly16 homozygotes experienced an improvement in PEFR during regular albuterol therapy.

Reproduced from Israel et al. (67).

One prospective, genotype stratified clinical trial analyzing $Gly^{16}Arg$ genotypes and responses to LABA treatment was performed by ACRN investigators, the Long-Acting Beta Agonist Response by Genotype (LARGE) trial. In the LARGE trial, 42 Arg^{16} homozygotes and 45 Gly^{16} homozygotes were randomized, in a cross-over fashion, to salmeterol or placebo in addition to ICS therapy for 18 weeks with ipratropium rescue inhaler therapy to minimize beta agonist exposure. At the end of the treatment periods both genotype groups experienced similar improvements in lung function; however, Gly^{16} homozygotes experienced a greater increase in bronchial reactivity to methacholine, a "bronchoprotective effect" that was not observed among Arg^{16} homozygotes and requires further investigation (74). A larger prospective, genotype-stratified, pharmacogenetic trial was performed by Bleecker et al., who randomized 179 Arg^{16} homozygotes, 182 $Gly^{16}Arg$ heterozygotes, and 183 Gly^{16} homozygotes to 16 weeks of salmeterol with ICS or salmeterol monotherapy. That trial is important because it showed similarities in lung function response between $Gly^{16}Arg$ genotypes during LABA therapy with or without concomitant ICS. The study showed that the absence of a $Gly^{16}Arg$ genotype effect is unrelated to concomitant ICS therapy, which acts synergistically with LABA during combination therapy, as depicted in Figure 10 (32,33,75).

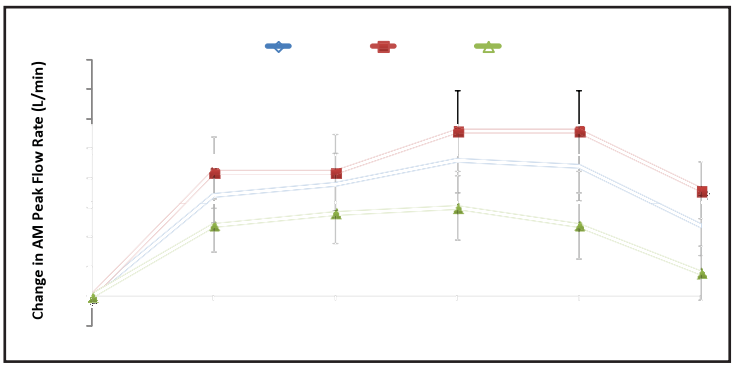

Figure 10 - Morning (AM) PEFR by genotype for subjects randomized to salmeterol monotherapy in a prospective, genotype-stratified trial.

Reproduced from Bleecker et al. (74).

Current evidence consistently suggests that a variation in *ADRβ2* at the Gly[16]Arg locus is a determinant for the response to acute and chronic SABA therapy. Despite these observations, this variant does not determine response to LABA therapy with or without concomitant ICS therapy. The lack of a genotypic effect for a common variant such as Gly[16]Arg suggests that if an effect does exist, it is either on an outcome so rare that studies to date have been underpowered to detect it or an effect that depends on interactions with other variants in *ADRβ2* and pathway-related genes to determine the response to LABA therapy. For example, a common coding variant in a pathway-related gene encoding for adenylyl cyclase type 9 (ADCY9) has also been shown to affect the response to albuterol among children with asthma treated with ICS (37).

It is also possible that rare variants that might have larger effects on phenotypes determine rare responses to LABA therapy, including life-threatening exacerbations. A rare variant identified within *ADRβ2*, Thr[164]Ile, is associated with diminished receptor coupling to the pathway-related Gs protein, as demonstrated by a 50% decrease in adenylyl cyclase activity and results in a 4-fold decrease in receptor binding affinity for isoproterenol and epinephrine. In addition, pharmacogenetic pathway analyses in larger asthma populations utilizing genome-wide approaches and detailed resequencing will be necessary in order to identify common and rare variants along the β_2-adrenergic receptor pathway that determine the risk for rare but serious adverse responses to beta agonists. This might help to identify the small subset of asthma patients that might benefit from alternatives to long-acting bronchodilator therapies, for example, long-acting muscarinic antagonists (76).

FINAL CONSIDERATIONS: ALTERNATIVE PATHWAYS AND THE FUTURE OF PHARMACOGENETICS

Pharmacogenetic approaches have the promise of determining drug responses to therapies that are expansive or have potential side effects, making it necessary to determine the subpopulation that is responsive to a given therapy. Molecular inhibitors and monoclonal antibodies have been designed to target alternative inflammatory pathways for the management of asthma. Pitrakinra is a recombinant IL-4 variant that inhibits binding of IL-13 and IL-4 to the IL-4α receptor subunit to attenuate Th2 lymphocyte-mediated allergic inflammation. In a randomized, placebo-controlled trial of patients with atopic asthma, inhaled pitrakinra treatment was associated with improvements in antigen-induced airway hyperresponsiveness with effects determined by coding variants in the gene encoding for the IL4α receptor subunit (*IL4RA*) (77,78). More recently, in a large phase IIb clinical trial, Slager et al. showed that response to this IL-4α antagonist was determined by specific *IL4RA* genotypes. Approximately one third of severe asthma patients responded in a significant dose-response paradigm to this drug, while the remaining two thirds of the population had no response (79). Thus, the nonresponsive asthma patients should not receive this therapy and *IL4RA* genotypes could serve as a useful therapeutic biomarkers. There are multiple experimental biologic therapies currently under development for the management of severe asthma. Therefore, it will become increasingly important to design pharmacogenetic studies to identify the subset of asthma patients that would benefit from these novel therapies.

Review of the pharmacogenetics of asthma therapies and their respective pathways, shows that genomics research can contribute to the personalized medicine of the future where genetic determinants could predict clinical responses, whether beneficial or adverse. Our understanding of the pharmacogenetics of the leukotriene, corticosteroid, and β_2-adrenergic receptor pathways have improved our understanding of the genetic determinants of clinical response but are limited by the inherent weaknesses of candidate gene analyses in smaller cohorts. Most of the current analyses are limited to small clinical trial populations with a biased candidate gene approach with SNPs that might "tag" for causative variants, requiring replication in larger trial cohorts. Future clinical trials will need to analyze larger populations using genome-wide association approaches in order to identify new, unbiased pharmacogenetic targets that predict drug response. In addition, resequencing of candidate genes or whole exome sequencing can identify rare variants or other forms of gene variation such as polynucleotide insertions or deletions that have the potential for strong genetic effects on drug response phenotypes. Some of the genetic variants we have discussed have low allele frequencies and are suited for analysis with a prospective, genotype-stratified design ensuring that individuals with less common risk variants are well represented in a clinical trial population. In order for personalized medicine to be translated to clinical medicine, it is important to replicate pharmacogenetic discoveries with analyses of larger clinical trial cohorts and, when appropriate, prospective, genotype-stratified pharmacogenetic studies.

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