



Asthma pharmacogenetics: responding to the call for a personalized approach

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Purpose of review

Asthma is a chronic, complex disease that is treated with a combination of different therapies. However, interindividual variability in clinical responses to different therapies complicates asthma management. A personalized approach to asthma management could identify appropriate responders to specific agents or those that might be at an increased risk for adverse responses.

Recent findings

Pharmacogenetic studies of genes from the leukotriene, glucocorticoid, and beta₂-adrenergic receptor pathways have improved our understanding of how gene variation determines therapeutic responses to different classes of antiasthma therapies. Such studies have previously been limited to retrospective analyses of candidate genes in the leukotriene, glucocorticoid, and beta₂-adrenergic receptor pathways in trial cohorts. However, prospective genotype-stratified trials in asthma have recently been done and recent genome-wide association studies have identified novel pharmacogenetic loci.

Summary

It will be important to replicate previous genotypic associations in large clinical trial cohorts as future pharmacogenetic studies continue to focus on genome-wide approaches and the study of novel therapeutic pathways. This review of the pharmacogenetics of asthma highlights the contributions of genomics research to the future of personalized medicine in asthma and draws attention to the role of genetic biomarkers in predicting clinical responses to specific therapies.

Keywords

asthma, genes, pharmacogenetics, response heterogeneity, single nucleotide polymorphism

INTRODUCTION: THE CALL FOR PERSONALIZED APPROACHES IN ASTHMA

Asthma is a common inflammatory disease of the airways that affects more than 300 million worldwide [1]. It is a chronic and complex disease determined by the interaction of different heritable and environmental factors, all of which contribute to marked interindividual (between persons) heterogeneity in phenotypic expression [2]. A combination of different therapies are available for the treatment of asthma and include inhaled and systemic corticosteroids, inhaled beta₂-(β₂) adrenergic receptor agonists (beta-agonists), leukotriene modifiers, inhaled anticholinergics, theophylline, and monoclonal antibodies that bind immunoglobulin E.

Individuals with asthma show marked interindividual variability in clinical drug responses to these therapies. This contributes to two problems that complicate the management of asthma. First,

up to 30% of asthmatic patients experience poor symptom control and recurrent exacerbations despite treatment with multiple asthma therapies; this results in greater morbidity and a higher financial burden among these 'refractory' asthmatic patients compared with those with milder disease [3–5]. Second, specific asthma therapies, inhaled beta-agonists in particular, have been associated with adverse events that may be life-threatening

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Curr Opin Allergy Clin Immunol 2013, 13:399–409

DOI:10.1097/ACI.0b013e3283630c19

KEY POINTS

- Asthma is a chronic, complex disease in which interindividual variability in clinical responses to different therapies complicates management providing the rationale for a personalized approach to identify both appropriate responders to specific agents and those that might be at an increased risk for adverse responses.
- Pharmacogenetic studies in asthma were previously limited to candidate genes in the leukotriene, glucocorticoid, and β_2 -adrenergic receptor pathways in small trial cohorts; however, investigators have recently performed genome-wide association studies to identify novel pharmacogenetic loci.
- Pharmacogenetics has the potential to identify genetic determinants of response to novel antiasthma therapies that target alternative inflammatory pathways such as pitrakinra and the interleukin-4 inflammatory pathway.
- It will be important to replicate previous discoveries in large clinical trial cohorts with candidate gene analyses, genome-wide approaches, and genotype-stratified pharmacogenetic studies before we will be able to translate them into personalized approaches.

[6,7]. These issues provide the rationale for a personalized approach to asthma management with a goal to develop molecular biomarkers to identify both appropriate responders to specific agents and those that might be at an increased risk for adverse responses. Below, we will discuss the rationale for pharmacogenetics research in asthma and how it has been applied to different therapeutic pathways to address the dilemmas of asthma management.

THE RATIONALE FOR PHARMACOGENETICS IN ASTHMA

Pharmacogenetics is the field that examines the role of genetic variability in determining interindividual phenotypic responses to pharmacologic therapies. Pharmacogenetics is essentially a gene–environment interaction in which the environmental exposure is a specific therapy. Individuals with asthma show marked interindividual variability in clinical responses to different antiasthma therapies, whereas intraindividual (within person) variability in responses to different therapeutic agents is often low, suggesting that heritable or genetic factors play an important role [8–10]. Based on these observations, genetic variation could potentially be used to identify responders, nonresponders, or those at risk for adverse responses to specific agents: this is the primary rationale for pharmacogenetic studies in asthma.

Pharmacogenetic studies in asthma have been primarily limited to pharmacodynamic endpoints due to the nature of the retrospective analyses employed in the majority of studies. In these retrospective pharmacogenetic studies, investigators analyzed DNA from participants for genotypic associations with predetermined clinical endpoints such as forced expiratory volume in one second (FEV_1), peak expiratory flow rates (PEFRs), or asthma exacerbations after the completion of a clinical trial. Retrospective pharmacogenetic studies are important for gene variant discovery and can employ analytical approaches such as candidate gene analyses and genome-wide association studies (GWASs). A minority of pharmacogenetic studies have employed a prospective, genotype-stratified study design in which DNA was collected from trial participants before randomization and asthma patients were allocated to treatment or placebo groups based on predefined genotypes. The strength of a prospective, genotype-stratified design lies in its statistical power to analyze less common genetic variants because an adequate sample size has been recruited with a predefined risk genotype. Below we will describe how these pharmacogenetic approaches have been applied to the different therapeutic pathways which are the basis for current asthma treatment: the glucocorticoid, cysteinyl leukotriene, and β_2 -adrenergic receptor pathways (Table 1) [11–21,22^{••},23^{••},24–45].

VARIABILITY OF DIFFERENTIAL RESPONSES TO INHALED GLUCOCORTICOIDS AND LEUKOTRIENE MODIFIERS

The molecular mechanisms that determine refractory asthma remain unclear; however, it is plausible that a subset of asthmatic patients are either non-responsive or experience negative therapeutic responses to specific therapies. Clinical trials consistently show that inhaled corticosteroids (ICSs) have greater beneficial effects on asthma symptom control and lung function compared with leukotriene receptor antagonists; however, these observations are based on mean responses and do not account for interindividual variability [46–48]. Among asthma patients randomized to an ICS or the leukotriene modifier, montelukast, a subset experienced an improvement in FEV_1 with either therapy alone, whereas some patients experienced either no response or a negative response to either treatment [46,49]. The variability in responses to ICS and leukotriene modifiers emphasizes an important rationale for pharmacogenetic research to identify therapies that are efficacious, ineffective, or even

Table 1. Pharmacogenetic candidate genes in asthma

Drug classes	Gene	Associated variants	Study designs	Asthma cohort sizes	Pharmacogenetic effects [ref]
Leukotriene receptor modifiers: 5-lipoxygenase inhibitors (ABT-761 and zileuton)	<i>ALOX5</i>	Promoter Repeat, rs892690, rs2029253, rs2115819	Retrospective analysis	114, 577	FEV ₁ response [11,13]
	<i>LTC4S</i>	rs272431	Retrospective analysis	577	FEV ₁ response [13]
	<i>MRP1</i>	rs215066, rs119774	Retrospective analysis	577	FEV ₁ response [13]
Cysteinyl leukotriene antagonists (montelukast)	<i>ALOX5</i>	Promoter Repeat, rs2115819	Retrospective analysis	61, 174	FEV ₁ response [12,14]
	<i>MRP1</i>	rs119774	Retrospective analysis	61	FEV ₁ response [14,15]
	<i>LTC4S</i>	rs730012	Retrospective analysis	61	Exacerbation risk [14]
	<i>LTA4H</i>	rs266845	Retrospective analysis	61	Exacerbation risk [14]
	<i>ADRB2</i>	rs1042713 (Gly16Arg)	Prospective, genotype-stratified trial	62	Preference for montelukast or LABA as add-on to ICS [39]
Inhaled glucocorticoids: (fluticasone, budesonide, flunisolide, triamcinolone)	<i>CRHR1</i>	rs242941, rs1876828, rs2236647, rs6591838,	Retrospective analysis	1117	FEV ₁ response [16]
	<i>STIP1</i>	rs1011219	Retrospective analysis	382	FEV ₁ response [17]
	<i>TBX21</i>	rs2240017 (His33Glu)	Retrospective analysis	53, 701	Bronchoprotection [18,19]
	<i>GLCCI1</i>	rs37972 = rs37973, rs3127412, rs6456042,	Genome-wide association study	1053	FEV ₁ response [22 ^{***}]
	<i>T Gene</i>	rs3099266, rs2305089	Genome-wide association study	815	FEV ₁ response [23 ^{***}]
	<i>CYP3A4</i>	CYP3A4*22	Retrospective analysis	413	Asthma control [24]
	<i>ADCY9</i>	rs2230739 (Met772Ile)	Retrospective analysis	86	Long-term FEV ₁ response [21]
	<i>CRHR2</i>	rs7793837	Retrospective analysis	1186, 2145	Acute FEV ₁ bronchodilation [44,45]
Inhaled β2-adrenergic receptor agonists: short-acting beta-agonists (albuterol)	<i>ADCY9</i>	rs2230739 (Met772Ile)	Retrospective analysis	436, 2145	Acute FEV ₁ bronchodilation [20,44]
	<i>ADRB2</i>	rs1042713 (Gly16Arg)	Retrospective and prospective,	16, 195, 269, 684, 707	Acute FEV ₁ bronchodilation [25–29]
			Genotype-stratified studies	78, 115, 190	Long-term PEFr response [30–32]
	<i>ARG1</i>	rs2781659, rs2781667	Retrospective analysis	200, 749, 1073, 2145	Acute FEV ₁ bronchodilation [40–42,44]
	<i>ARG2</i>	rs17249437, rs3742879	Retrospective analysis	200	Acute FEV ₁ bronchodilation [41]
	<i>NOS3</i>	rs1799983 (Glu298Asp)	Retrospective analysis	81	Acute FEV ₁ bronchodilation [43]
	<i>SPATS2L</i>	rs295137	Genome-wide association study	2145	Acute FEV ₁ bronchodilation [44]

Table 1 (Continued)

Drug classes	Gene	Associated variants	Study designs	Asthma cohort sizes	Pharmacogenetic effects [ref]
Long-acting beta-agonists (salmeterol and formoterol)	ADCY9	rs2230739 (Met772Ile)	Retrospective analysis	86	Long-term FEV ₁ response [21]
			Retrospective and prospective,	87, 115, 183, 544, 2655	No effect on PEFR response [31,34 [■] ,35 [■] ,36,37]
	ADRB2	rs1042713 (Gly16Arg)	Genotype-stratified studies	87, 152	Bronchoprotection [37,38]
			Prospective, genotype-stratified trial	62	Preference for montelukast or LABA as add-on to ICS [39]

Candidate genes discussed in this review by drug class, associated polymorphisms (by rs number and coding change if relevant), study design, population size of each study cohort, and phenotype for which a pharmacogenetic effect has been detected. [ref], reference cited in text; ICS, inhaled corticosteroid; LABA, long-acting beta-agonist; PEFR, peak flow rate.

harmful for individual asthmatic patients. Future personalized approaches would then identify asthmatic patients less responsive to ICS who might benefit from an alternative, first-line controller therapy such as a leukotriene modifier or vice versa.

Pharmacogenetics of the cysteinyl leukotriene pathway

The cysteinyl leukotriene pathway is an important pathway in the pathogenesis and treatment of asthma. Cysteinyl leukotrienes are synthesized by a cascade of enzymes initiated by the conversion of arachidonic acid to leukotriene A₄ by 5-lipoxygenase (5-LO) encoded by the *ALOX5* gene. Subsequent steps in this pathway result in the synthesis of different leukotrienes (leukotriene A₄ hydrolase encoded by *LTA4H* and leukotriene C₄ synthase by *LTC4S*) that are transported to the extracellular space by multidrug resistance protein 1 (*MRP1*) to activate cysteinyl leukotriene receptors (*CYSLTR1* and *CYSLTR2*).

One of the earliest pharmacogenetic studies in asthma was a retrospective analysis of a clinical trial in which 114 participants were randomized to a 5-LO inhibitor, ABT-761, or placebo. An *ALOX5* promoter tandem repeat polymorphism was associated with FEV₁ response to ABT-761 treatment in this cohort, a genotypic effect also observed in 61 asthmatic patients treated with the cysteinyl leukotriene antagonist, montelukast [11,12]. In addition, an analysis of cysteinyl leukotriene pathway genes in 577 asthma patients randomized to another 5-LO inhibitor, zileuton, demonstrated

three intronic single nucleotide polymorphisms (SNPs) in *ALOX5* (rs892690, rs2029253, and rs2115819), one SNP in *LTC4S* (rs272431), and two SNPs in *MRP1* (rs215066 and rs119774) associated with FEV₁ response to zileuton treatment [13].

Another analysis of leukotriene pathway-related genes in 61 patients treated with montelukast demonstrated SNPs in *ALOX5* (rs2115819) and *MRP1* (rs119774) associated with FEV₁ percentage change during treatment (Fig. 1) [14], whereas SNPs in *LTC4S* (rs730012) and *LTA4H* (rs2660845) were associated with risk of exacerbation. The beneficial responses were primarily observed among those who inherited the less common or minor allele of SNPs in *ALOX5*, *MRP1*, and *LTC4S* [14]. A similar genotypic effect of *ALOX5* gene variants was also observed in a larger cohort of 174 patients treated

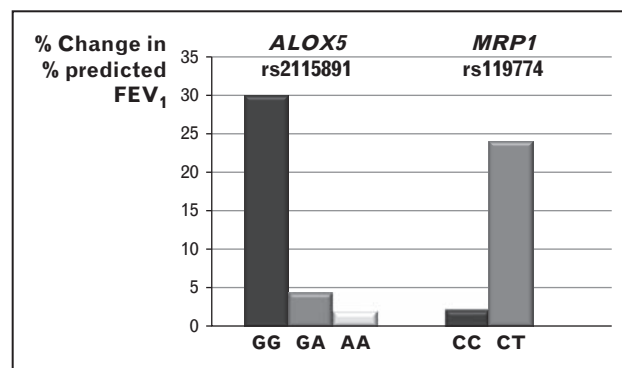


FIGURE 1. Percentage change in forced expiratory volume in 1 second (FEV₁) predicted over baseline after 6 months of montelukast treatment by *MRP1* rs119774 and *ALOX5* rs2115819 genotypes. Reproduced from [14].

with montelukast; however, further study of the therapeutic role of gene variation throughout the cysteinyl leukotriene pathway will require replication in larger cohorts or genotype-stratified pharmacogenetic studies powered for the risk genotypes [14,15].

Pharmacogenetics of the glucocorticoid pathway

The pharmacogenetics of the glucocorticoid pathway is based on analyses of genes from a complex pathway consisting of the glucocorticoid biosynthetic pathway, the cytosolic receptor heterocomplex, and chaperone proteins. Glucocorticoids activate receptor-chaperone complexes that translocate into the nucleus to repress the transcription of pro-inflammatory genes and enhance the transcription of anti-inflammatory genes [50]. Among the earliest pharmacogenetic studies of corticosteroids in asthma was a candidate gene analysis of the corticotropin-releasing hormone (*CRHR1*) gene in 1117 asthma patients randomized to ICS treatment in either the Childhood Asthma Management Program (CAMP), an 'Adult Study', or in Asthma Clinical Research Network (ACRN) trial cohorts. Two SNPs in *CRHR1* (rs242941 and rs1876828) were associated with changes in FEV₁ with ICS treatment [16]. Subsequently, an analysis of genes coding for the glucocorticoid receptor heterocomplex in the Adult Study cohort identified three SNPs within the gene coding for heat shock organizing protein, *STIP1* (rs2236647, rs6591838, and rs1011219), associated with FEV₁ response to the ICS, flunisolide [17].

An important aspect of the pharmacogenetics of the corticosteroid pathway is its interaction with other gene pathways [51,52]. *TBX21* encodes the T-box expressed in T-cell transcription factor that influences naive T-lymphocyte development through induction of T-helper (TH₁) cells and the suppression of TH₂ cells. A *TBX21* nonsynonymous SNP, His³³Glu, was associated with improvements in bronchial hyperresponsiveness or 'broncho-protection' in response to ICS in individuals from the CAMP cohort, a finding also observed in an independent Korean population [18,19]. *ADCY9* encodes adenylyl cyclase type 9, an enzyme in the canonical β_2 -adrenergic receptor pathway that contains a nonsynonymous SNP, Met⁷⁷²Ile, associated with bronchodilator response to the short-acting beta-agonist (SABA), albuterol, during 4 years of ICS treatment [20]. The interaction between the Met⁷⁷²Ile variant and ICS treatment was also reproduced in a Korean population [21].

Genome-wide approaches have more recently been used to identify novel pharmacogenetic determinants of ICS response. The first such study was a family-based GWAS in 118 probands from CAMP with replication in 935 asthma patients from four additional cohorts. A SNP in the promoter of the glucocorticoid-induced transcript 1 (*GLCCI1*), rs37972, was associated with lung function response to ICS, with homozygotes for the wild-type allele (CC) showing a consistently greater FEV₁ response to ICS compared with homozygotes for the variant allele (TT) resulting in a pooled *P* value of 0.0007 in the four replication cohorts. In a complementary in-vitro study [22²²], cells transfected with the minor allele of a strongly correlated promoter SNP, rs37973, resulted in reduced luciferase reporter activity. A second GWAS was performed in 408 asthma patients randomized to ICS treatment from CAMP, ACRN, and the Childhood Asthma Research and Education (CARE) network with replication in another 407 asthma

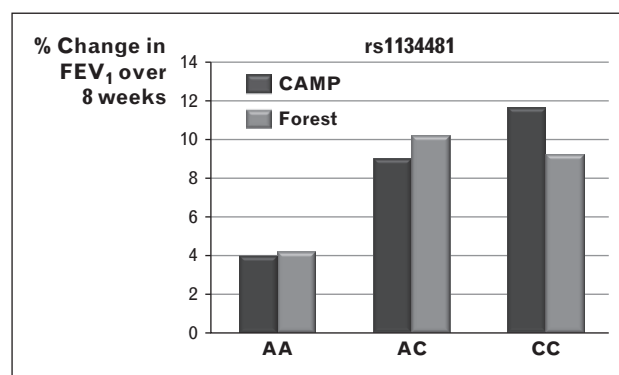


FIGURE 2. A genome-wide association study identifies the *T* gene as a pharmacogenetic loci for inhaled corticosteroid response. Tantisira *et al.* performed a genome-wide association study (GWAS) in 408 asthma patients randomized to inhaled corticosteroid (ICS) treatment from four different trial networks with replication in another 407 asthma patients. Two single nucleotide polymorphisms (SNPs) in the *T* gene (rs3127412 and rs6456042) had nominal associations with forced expiratory volume in one second (FEV₁) percentage response to ICS after replication; however, detailed genotyping of the *T* gene demonstrated additional strongly linked SNPs (rs3099266, rs1134481, rs2305089) within potentially functional genomic regions of this gene associated with ICS response. The strongest observed association was with a SNP in the 3' untranslated region of the *T* gene, rs1134481, in the Childhood Asthma Management Program cohort (*P*=0.0009) and the Forest trial cohort (*P*=0.01). This figure demonstrates percentage change in FEV₁ after 8 weeks of ICS treatment by *T* gene rs1134481 genotypes. Reproduced from [30²²].

patients. Two SNPs in the *T* gene (rs3127412 and rs6456042) were nominally associated with lung function response to ICS; however, detailed genotyping of the *T* gene demonstrated additional, strongly linked SNPs (rs3099266, rs1134481, rs2305089) within potentially functional genomic regions associated with ICS response (Fig. 2) [23²⁴].

A recent pharmacogenetic study is one of the few to evaluate the effects of gene variation that potentially alters drug metabolism (i.e. pharmacokinetics) on treatment response in asthma. This study was a recent retrospective analysis of 413 asthmatic children treated with an ICS who were genotyped for nine SNPs in *CYP3A4*, *CYP3A5*, and *CYP3A7*. In the subset of children treated with inhaled fluticasone, those with the *CYP3A4**22 allele demonstrated a significant improvement in asthma control during treatment compared with those without this allele; however, the number of children with this beneficial allele was small ($n = 20$) [24]. In addition, an asthma susceptibility locus, *ORDML3* (rs 2872507), has recently been associated with lung function response to ICS in a European population of asthmatic children [53]. Novel pharmacogenetic loci in *GLCCI1* and the *T* gene likely account for a small proportion of the observed variability of therapeutic responses to ICS, while genetic loci that determine drug metabolism and disease susceptibility may also contribute to therapeutic responses. These pharmacogenetic findings will require replication in larger trial cohorts or genotype-stratified pharmacogenetic studies to determine whether these variants have additive effects or are independent predictors of therapeutic responses.

DIFFERENTIAL RESPONSE AND ADVERSE EVENTS ASSOCIATED WITH INHALED BETA-AGONIST THERAPY

Surveillance studies and meta-analyses have elicited concerns for an increased risk of life-threatening exacerbations and death in those treated with long-acting beta-agonists (LABAs) resulting in a boxed warning from the Food and Drug Administration (FDA) [6,7,54]. In contrast, smaller, placebo-controlled clinical trials and meta-analyses have not shown an increased risk for life-threatening adverse events, and demonstrate that treatment with a LABA in combination with an ICS provides beneficial effects on exacerbation rates and symptom control [55–58]. The concern for adverse responses to LABA remains the subject of intense debate and controversy and will be evaluated in over 40 000 asthma patients in a LABA safety study mandated by the FDA [59,60]. Pharmacogenetic studies of inhaled

beta-agonists in asthma have the potential to identify those at increased risk of developing adverse effects.

Pharmacogenetics of the beta₂-adrenergic receptor pathway

The β_2 -adrenergic receptor activates a complex G-protein-coupled receptor pathway to mediate airway smooth muscle relaxation [20]. *ADRB2* is the most intensively studied gene in asthma pharmacogenetics and contains a common coding variant in the 16th amino acid position that codes for glycine or arginine, Gly¹⁶Arg, the most studied of over 49 *ADRB2* polymorphisms identified in asthma patients [61,62]. *In vitro*, the Gly¹⁶ allele results in enhanced receptor downregulation when compared with the Arg¹⁶ allele in Chinese hamster fibroblasts and human airway smooth muscle cells [63,64].

Early pharmacogenetic studies in two cohorts of asthmatic children demonstrated that Arg¹⁶ homozygotes experienced greater FEV₁ bronchodilation in response to a one-time administration of albuterol compared with Gly¹⁶ homozygotes, a genetic effect that has been replicated in smaller asthma cohorts including other ethnic groups such as Puerto Ricans [25–27]. Two retrospective pharmacogenetic studies [28,29] of regular, long-term albuterol therapy have also demonstrated that Arg¹⁶ homozygotes experienced a decline in PEFR, whereas Gly¹⁶ homozygotes did not experience a change in PEFR during regular albuterol treatment.

The ACRN Beta Agonist Response by Genotype (BARGE) trial prospectively investigated the genotypic effects of Gly¹⁶Arg during regular albuterol treatment with a genotype-stratified study design. Thirty-seven Arg¹⁶ homozygotes and 41 Gly¹⁶ homozygotes were randomized to regular albuterol or placebo with ipratropium as a rescue inhaler for 16 weeks with cross-over to the alternative therapy. During regular albuterol treatment, Arg¹⁶ homozygotes did not experience a change in PEFR and experienced deterioration of asthma symptom scores and rescue inhaler use, whereas Gly¹⁶ homozygotes experienced improvements in these endpoints (Fig. 3) [30].

Pharmacogenetic studies have also investigated whether Gly¹⁶Arg has genotypic effects on regular long-term LABA therapy including a small retrospective analysis of two ACRN trials that suggested adverse effects on PEFR, and asthma control among Arg¹⁶ homozygotes compared with Gly¹⁶ homozygotes during treatment with salmeterol [29,31]. Subsequently, large retrospective analyses of three different clinical trial cohorts by Bleecker *et al.* [32,33] failed to show significant differences in

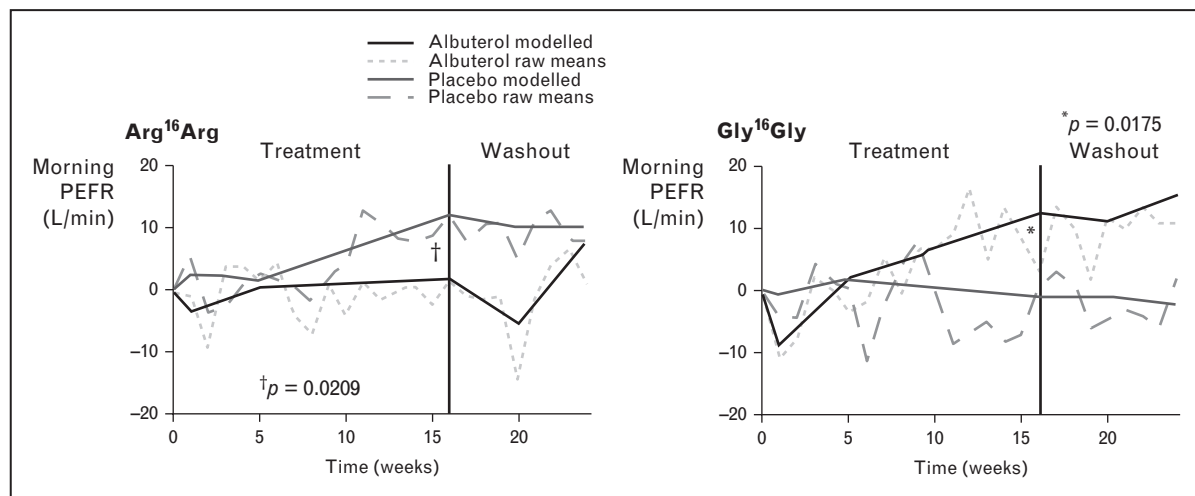


FIGURE 3. A prospective, genotype-stratified trial of Gly¹⁶Arg genotypes and regular short-acting beta-agonist treatment response. The ACRN Beta Agonist Response by Genotype (BARGE) trial was a prospective, genotype-stratified, placebo-controlled, cross-over trial in which 37 Arg¹⁶ homozygotes and 41 Gly¹⁶ homozygotes were randomized to 16-week treatment with regular albuterol or placebo with both groups receiving ipratropium as a rescue inhaler to minimize beta-agonist exposure throughout the trial. Arg¹⁶ homozygotes experienced no change in peak expiratory flow rate (PEFR) during albuterol treatment; however, PEFR improved during placebo treatment. On the contrary, Gly¹⁶ homozygotes experienced an improvement in PEFR during regular albuterol therapy. Reproduced from [49].

PEFR, exacerbations, or rescue inhaler between Gly¹⁶Arg genotypes during LABA treatment.

Two prospective genotype-stratified trials, the ACRN Long-Acting Beta Agonist Response by Genotype (LARGE) Trial and a trial by Bleeker *et al.* also failed to show differences in PEFR responses or exacerbation rates between Gly¹⁶Arg genotypes during LABA treatment, independent of whether LABA was administered as a monotherapy or in

combination with ICS therapy (Fig. 4) [34[■],35[■]]. However, the LARGE study did demonstrate trends of differential treatment response between African-American patients of different Gly¹⁶Arg genotypes and increased methacholine airway hyperresponsiveness in Arg¹⁶ homozygotes compared with Gly¹⁶ homozygotes after treatment with LABA, a 'bronchoprotective effect' previously observed in 152 asthma patients [35[■],36].

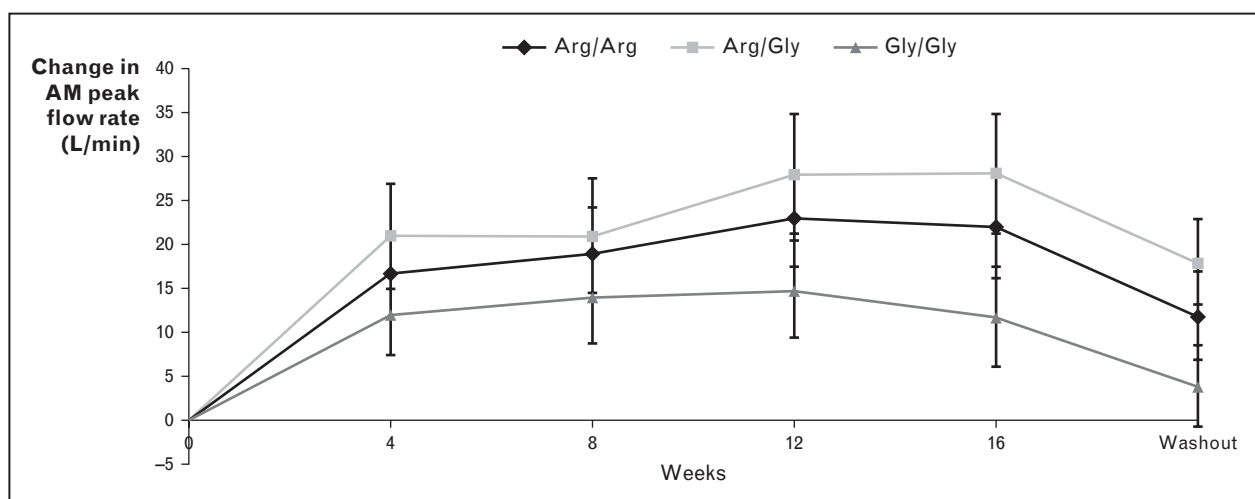


FIGURE 4. A prospective, genotype-stratified trial demonstrates that Gly¹⁶Arg genotypes do not determine peak flow response to long-acting beta-agonist therapy. Prospective, genotype-stratified trial by Bleeker *et al.* Outcome of morning (AM) peak flow rate by Gly¹⁶Arg genotype for individuals randomized to salmeterol monotherapy (error bars represent SEM). Reproduced from [53[■]].

A recent prospective pharmacogenetic study [37] of 62 Arg¹⁶ homozygote asthma patients demonstrated improved symptom control with montelukast treatment compared with treatment with salmeterol. Despite the fact that this study [38] did not include a comparison group with the alternative genotype (i.e. Gly¹⁶ homozygotes), these findings were the focus of a BBC news article entitled 'Spit test "improves" asthma care' illustrating that this aspect of asthma pharmacogenetics remains an important topic of public interest.

It is plausible that uncommon, yet severe, adverse events such as life-threatening exacerbations or death in response to LABA therapy depend on rare genetic variants with strong effects. For example, a rare *ADRB2* variant located at codon 164, Thr¹⁶⁴Ile, has been associated with impaired receptor ligand binding and coupling to G_s protein in response to different SABAs such as isoproterenol and albuterol [39,65]. These in-vitro effects have also been observed for LABAs and also include impaired binding of salmeterol to its receptor 'exosite' [65]. In a recent cross-sectional analysis of over 60 000 individuals from two population studies, the rare Ile¹⁶⁴ variant was associated with reduced lung function and airflow obstruction; however, the impact of rare *ADRB2* variants, such as Thr¹⁶⁴Ile, on therapeutic responses to beta-agonist remains to be studied in an asthma trial cohort [66]. A variable length polynucleotide cytosine repeat polymorphism (poly-C repeat) in the 5' untranslated region of *ADRB2* was recently genotyped in 2192 asthma patients randomized to LABA and ICS combination therapy; however, no genotypic effects were observed on lung function response or incidence of severe exacerbations during treatment. Of the 13 poly-C alleles of varying lengths identified in this population, six occurred at a frequency of greater than 5% indicating that over half of these alleles are rare variants with the potential for strong genotypic effects [67].

Adverse responses to beta-agonist therapy might depend on gene–gene interactions within molecular pathways that mediate bronchodilation such as *ADCY9*, a gene in the canonical β_2 -adrenergic receptor pathway with variation associated with albuterol bronchodilator response during ICS treatment [20]. An analysis of 111 candidate genes within the glucocorticoid and β_2 -adrenergic receptor pathways in 209 probands from CAMP and 864 asthma patients from two trial cohorts identified a SNP in the arginase 1 gene (*ARG1*), rs2781659, significantly associated with albuterol bronchodilation [68]. Another *ARG1* SNP and two SNPs in *ARG2* were also associated with albuterol bronchodilator response in 200 asthmatic patients from a Dutch

longitudinal study [69]. In addition, a haplotype analysis of four *ARG1* SNPs in 749 patients from three trial cohorts demonstrated three major *ARG1* haplotypes associated with albuterol bronchodilator response [40]. Arginase 1 and 2 metabolize L-arginine, a natural substrate for nitric oxide synthase, which produces the endogenous bronchodilator nitric oxide [41]. Recently, a nonsynonymous SNP in the gene encoding for endothelial nitric oxide synthase (*NOS3*), Asp²⁹⁸Glu was associated with lung function response to LABA and ICS combination therapy in 81 asthmatic children [42].

A recent GWAS of 1622 asthma patients from six clinical trial cohorts has identified a SNP in the 5' untranslated region of the Spermatogenesis associated, serine-rich 2-like gene (*SPATS2L*), rs295137, associated with acute bronchodilator response to SABA. This was replicated in 501 individuals from the NIH NHLBI Severe Asthma Research Program (SARP) for a pooled *P* value of 7.7×10^{-7} . *In vitro*, miRNA knockdown of *SPATS2L* in human airway smooth muscle cells resulted in increased β_2 -adrenergic receptor expression [70[■]]. The investigators also identified SNPs associated with SABA bronchodilator response in *ADCY9*, *CRHR2*, and *ARG1* using genotypes from the primary GWAS replicating prior pharmacogenetic candidate gene analyses of these β_2 -adrenergic receptor pathway-related genes [20,68,70[■],43]. The impact of rare *ADRB2* variants and pathway-related gene variation on regular LABA therapy has yet to be investigated in trial cohorts to determine whether they have additive effects or are independent predictors of adverse responses during long-term beta-agonist exposure.

PITFALLS IN INTERPRETING PHARMACOGENETIC ASSOCIATIONS

Many of the pharmacogenomic associations detected in large populations have only small effects on response to therapy. However, even gene variation with large effects on pharmacologic response (i.e. Gly¹⁶Arg) may not produce an entirely uniform effect on response to a therapy. Additional sources of variability need to be considered that may confound resulting in misleading associations or introduce sufficient 'noise' to mask true associations. These sources of variability may be host factors, gene–gene interactions, or environmental effects. Host factors such as disease severity may have their own genetic determinants that introduce additional genetic variability.

Simultaneous genetic alterations may magnify or diminish an apparent pharmacogenomic effect. Such coincident polymorphisms can occur within the same gene as a haplotype or among different

genes resulting in gene–gene interactions. These interactions can be further modified in different admixed populations with varying ancestral genetic backgrounds that may not display the same associations with a particular set of genetic markers. For example, the Gly¹⁶ Arg in *ADRB2* predicts albuterol bronchodilator response in Puerto Rican but not Mexican populations [26].

Environmental effects may also affect differential response. For example, smoking modulates the response to ICS while *ADRB2* polymorphisms are differentially associated with airway hyper-responsiveness between smoking and nonsmoking populations [44,45]. In addition, specific environmental effects may occur in a particular ethnic group, perhaps due to cultural differences. Because these sources of variability may confound apparent pharmacogenetic associations, it is important to reconfirm apparent associations, especially those without clear functional correlates, in multiple populations.

FUTURE PHARMACOGENETIC APPROACHES

Pharmacogenetic studies in asthma were previously limited to candidate genes approaches in small populations; however, investigators have recently performed GWAS with replication in additional cohorts or cell-based models to validate the clinical impact of associated SNPs. Next-generation DNA sequencing is the next logical step in whole-genome and candidate gene analyses and identifies rare variants, polynucleotide insertion-deletions, or duplications that may be causative or co-inherited (i.e. in linkage disequilibrium) with associated SNPs.

Pharmacogenetics has large implications for drug development. It may permit the introduction and development of drugs for asthma that would have previously been abandoned due to potential toxicity in a subset of patients or a lack of efficacy in a study population. Pharmacogenetic approaches could potentially identify genetic determinants of response to novel antiasthma therapies. For example, the interleukin-4 (IL-4) inflammatory pathway mediates T-helper₂ lymphocyte-mediated allergic inflammation by binding and activating the IL-4 α receptor subunit (encoded by *IL4RA*), which is blocked by the molecular inhibitor, pitrakinra. Specific variants in *IL4RA* have been associated with beneficial effects on asthma exacerbations during pitrakinra treatment in a large phase IIb clinical trial [71]. Pharmacogenetic studies may identify those who will or will not benefit from expensive biologic therapies targeting novel

pathways currently being developed for the treatment of refractory asthma.

CONCLUSION

This review of the pharmacogenetics of antiasthma therapies highlights the contributions of genomics research to the future of personalized medicine wherein genetic biomarkers predict clinical responses to specific therapies, whether beneficial or adverse. Pharmacogenetic studies of genes from the leukotriene, glucocorticoid, and β_2 -adrenergic receptor pathways have improved our understanding of how gene variation determines therapeutic responses to different classes of antiasthma therapies. It is likely that in the future we will use panels of polymorphisms to ascertain relative risk-benefit ratios of particular therapeutic approaches for an individual patient. First, costs of genotyping must come down, and our understanding of how to utilize the wealth of genetic data and its correlation with pharmacologic outcomes must improve. As such, it will be important to replicate our pharmacogenetic discoveries in large clinical trial cohorts with candidate gene analyses, genome-wide approaches, and genotype-stratified pharmacogenetic studies if we hope to ultimately translate these discoveries into personalized approaches in clinical medicine.

Acknowledgements

None.

Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 455).

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