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Pharmacogenetics of the β 2-Adrenergic Receptor Gene

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Abstract

Asthma is a complex genetic disease with multiple genetic and environmental determinants contributing to the observed variability in response to common anti-asthma therapies. Asthma pharmacogenetic research has focused on multiple candidate genes including the β 2-adrenergic receptor gene (ADR β 2) and its effect on individual responses to beta agonist therapy. At present, knowledge about the effects of ADR β 2 variation on therapeutic responses is evolving and should not alter current Asthma Guideline approaches consisting of the use of short acting beta agonists for as-needed symptom based therapy and the use of a regular long-acting beta agonist in combination with inhaled corticosteroid therapy for optimal control of asthma symptoms in those asthmatics who are not controlled on inhaled corticosteroid alone. This approach is based upon studies showing a consistent pharmacogenetic response to regular use of short acting beta agonists (SABA) and less consistent findings in studies evaluating long acting beta agonist (LABA). While emerging pharmacogenetic studies are provocative and should lead to functional approaches, conflicting data with responses to LABA therapy may be caused by factors that include small sample sizes of study populations and differences in experimental design that may limit the conclusions that may be drawn from these clinical trials at the present time.

Introduction

Asthma is a common disease that affects greater than 300 million people worldwide.¹ It is a complex, chronic disease with multiple genetic and environmental determinants contributing to its heterogeneous phenotypic expression. Patients with asthma can present with varying degrees of airway inflammation and airflow obstruction that can be treated with different combinations of short-term relief and long-term controller medications including beta-2 adrenergic receptor agonists, corticosteroids (usually inhaled), leukotriene modifiers, cromones, and theophylline.

Asthma is heterogeneous in its responsiveness to common pharmacological therapies. Between 70 to 80% of patients with asthma have a variable response to common anti-asthma therapies, whether beneficial or detrimental.^{2,3,4} This variability in drug responses cannot be simply attributed to differences in patient adherence, suggesting that a proportion of this variance may be caused by pharmacogenetic factors.⁴⁻⁶ Pharmacogenetics represents a gene by environment interaction where the environment is the drug exposure and the result is the variation in

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individual response to pharmacologic therapy. (Figure 1) While additional factors such as interactions with other medications, co-morbid conditions, and age also effect the variability in response to anti-asthma therapies, it has been estimated that 20 to 95% of the variation in drug response may be attributed to heritable differences.^{4,5,6}

Background Issues: β 2-adrenergic Agonist Controversy

Among the anti-asthma therapies available today, β 2-adrenergic receptor agonists (e.g., beta agonists) are the most commonly prescribed therapeutic agents for the management of asthma and other obstructive pulmonary diseases.⁷ Two classes of inhaled β 2-adrenergic receptor agonists include the long-acting beta agonists [LABA: salmeterol and formoterol] and short-acting beta agonists [SABA: (albuterol)]. LABA therapy is used in conjunction with inhaled corticosteroid therapy (ICS) for the management of asthma during long-term maintenance therapy and SABA therapy is used for acute, as-needed bronchodilator symptom relief.⁷

While beta agonists are the most commonly used therapeutic agents in asthma, this drug class has been associated with adverse therapeutic events that have been primarily related to dosing regimens and potency of the specific agent. In the United Kingdom and other countries in which higher dose isoproterenol inhalers (a potent non-specific beta short acting beta agonist) were marketed, there was an increased asthma mortality that disappeared with the withdrawal of this preparation⁸. In the period between 1976 and 1989 in New Zealand fenoterol (a potent non-specific SABA) was marketed in a high dose and regular dose inhalers. Asthma mortality increased with this agents' release and decreased when it was taken off the market⁹⁻¹¹. Additional data showed that increased numbers of prescriptions of this drug and other SABA's increased the risk of mortality implying either a cause and effect relationship or that increased SABA use was a marker of poor asthma control¹²⁻¹⁴. Subsequently, Sears and colleagues showed that asthma control was worse with regular use of fenoterol¹² and the NIH Asthma Clinical Research Network Trial (ACRN) BAGS reported that asthma control did not improve with regular compared to as needed symptomatic use of the SABA albuterol¹⁵. Finally, two more recent studies have reported an increase for asthma related mortality (SNS)¹⁶ and respiratory deaths primarily in patients receiving LABA mono-therapy (SMART Study)¹⁷. While some meta analysis have reported increased risk with LABA¹⁸ therapy other meta analysis including a Cochrane analysis and a large case-control analysis have not shown adverse effects of LABA on major asthma exacerbations and life threatening events¹⁹⁻²¹. In addition a large number of clinical trials have shown that the addition of a LABA to an inhaled corticosteroid reduces exacerbations and improves asthma control.²²⁻²⁷ This background information on the potential adverse effects of beta agonist therapy is important because it emphasizes the heterogeneity in therapeutic responses to beta agonist therapy and the importance of understanding pharmacogenetic responses with beta agonist therapy in asthma.

Pharmacogenetics of the $ADR\beta 2$ Gene

Pharmacogenetics is the study of the role of genetic variability in determining individual responses to pharmacological therapies, such as beta agonists. (Figure 1) The goal of pharmacogenetic research to asthma is to characterize genetic determinants that will predict the likelihood that an individual with asthma will respond favorably or adversely to a given pharmacological agent such as beta-agonists. To date, most of asthma pharmacogenetic research has focused on SABA and LABA therapy, the β 2-adrenergic receptor and the gene encoding for the receptor, the β 2-adrenergic receptor gene or $ADR\beta 2$. Over the last 20 years genomic investigators have characterized genetic variants or polymorphisms throughout $ADR\beta 2$ and the contribution of those variants to asthma susceptibility and susceptibility to the adverse effects of LABA and SABA therapy. In this review, we will discuss the pharmacogenetics of $ADR\beta 2$ genetic polymorphisms from the molecular level to the clinical

findings and the role that genomic research has played in determining the role of *ADRβ2* genetic variation in the management of asthma with SABA and LABA therapy.

ADRβ2: The Gene

ADRβ2 was first sequenced approximately 20 years ago. *ADRβ2* is a small, intronless gene with one exon that encodes for a 413-amino acid, G-protein coupled receptor, the β2-adrenergic receptor.^{28,29} *ADRβ2* is an ideal candidate gene for the study of asthma genetics due to its location on chromosome 5q31: a region within the human genome consistently linked to asthma and related phenotypes (bronchial hyperresponsiveness (BHR), and serum IgE levels) through large family-based linkage studies in the Dutch, Caucasians, African Americans, and U.S. Hispanics.³⁰⁻³³ The linkage to asthma susceptibility within this region of the human genome has also been shown to have a gene-environment interaction with passive smoking exposure.³⁰

In 1992, a detailed mutational analysis of *ADRβ2* was reported by Reihaus et al who identified nine genetic variants or polymorphisms along the coding region of *ADRβ2*.³⁴ Four of these polymorphisms are single nucleotide changes in the genetic code referred to as single nucleotide polymorphisms (SNPs). These SNPs cause coding changes of the amino acid (AA) product at each codon or amino acid position relative to the start codon. Denoted as AA₁^{codon}AA₂ (AA₁ and AA₂ referring to the amino acid products of the two differing alleles) these SNPs include Gly¹⁶Arg, Gln²⁷Glu, Val³⁴Met, and Thr¹⁶⁴Ile. By definition, a single SNP and its resulting amino acid product determines an individual's genotype (e.g., Arg¹⁶ versus Gly¹⁶). However, more than one SNP evaluated as a group determines an individual's haplotype (e.g., Arg¹⁶/Gln²⁷). Gly¹⁶Arg and Gln²⁷Glu are two common *ADRβ2* SNPs in the general population that have been identified in all ethnic and racial groups screened to date with frequencies that vary between ethnic groups such as Caucasians and African-Americans, independent of asthma disease status. The Val³⁴Met and Thr¹⁶⁴Ile polymorphisms are exceedingly rare. No individuals homozygote for the less common allele at codon 164, Ile¹⁶⁴, have been identified to date due to the rare frequency of this allele.³⁵

ADRβ2 Pharmacogenetics at the Molecular Level

In vitro or cell-based genetic studies of *ADRβ2* have characterized the functional role of genetic variation within *ADRβ2* in the regulation of beta agonist-induced downregulation and desensitization of the β2-adrenergic receptor. Two *ADRβ2* polymorphisms, Gly¹⁶Arg and Gln²⁷Glu, have both been shown to influence the downregulation of the β2-adrenergic receptor in Chinese hamster fibroblasts and human airway smooth muscle cells.^{36, 37} In Chinese hamster fibroblasts, substitution of the amino acid combination or haplotype Arg¹⁶/Gln²⁷ with Gly¹⁶/Gln²⁷ or Gly¹⁶/Gln²⁷ (basically, changing Arg¹⁶ to Gly¹⁶) results in enhanced isoproterenol-induced downregulation of the β2-adrenergic receptor from 29% to 41% and 39%, respectively. Conversely, substitution of Arg¹⁶/Gln²⁷ with Arg¹⁶/Gln²⁷ (changing Gln²⁷ to Gln²⁷) results in resistance to isoproterenol-induced downregulation of the receptor.³⁶ In human airway smooth muscle cells the findings were similar: receptors with the Gly¹⁶ genotype underwent enhanced agonist-promoted downregulation while the Gln²⁷ genotype showed attenuation of agonist-promoted desensitization with isoproterenol.³⁷

In human airway smooth muscle cells, the effect of *ADRβ2* polymorphisms on beta agonist-induced acute and long term β2-adrenergic receptor desensitization were studied by measuring the effects of isoproterenol treatment on muscle cell relaxation and cyclic adenosine monophosphate (cAMP) levels. The Gln²⁷ allele (assumed to be Gly¹⁶/Gln²⁷) was associated with greater acute and long-term desensitization. Haplotype analysis of *ADRβ2* indicates that the Arg¹⁶ allele is almost always coinherited with the Gln²⁷ allele or that these polymorphisms are in "linkage disequilibrium" (in other words, Arg¹⁶/Gln²⁷, Gly¹⁶/Gln²⁷, and Gly¹⁶/Gln²⁷

are common haplotypes while Arg¹⁶/Gln²⁷ is rare).³⁸ In the human airway smooth muscle cell model, the Gly¹⁶/Gln²⁷ haplotype exhibited less acute and long-term desensitization than Gly¹⁶/Gln²⁷ virtually eliminating the effect of the Gly¹⁶ genotype.^{39,40}

Arg^{19(BUP)}Cys is a SNP located in the promoter region of *ADRβ2* (position -47 relative to the start codon) in the 5' leader cistron or the Beta Upstream Peptide (BUP), a 19 amino acid peptide that modulates receptor translation (Figure 1). The Gln²⁷Glu polymorphism's association with beta agonist-induced receptor desensitization may be explained, at least in part, by its association through linkage disequilibrium with Arg^{19(BUP)}Cys since Gln²⁷ is coinherited with Arg^{19(BUP)} and Gln²⁷ is coinherited with Cys^{19(BUP)}.^{34, 35} Studies of COS-7 cells transfected with Arg¹⁹Cys genotypes show that cells with the Cys^{19(BUP)} allele exhibit increased β2-adrenergic receptor translation. Interestingly, levels of the mRNA transcripts between genotypes were similar indicating that Arg^{19(BUP)}Cys only regulates receptor translation, not transcription.⁴¹

Drysdale and co-workers investigated the interaction of multiple *ADRβ2* polymorphisms on transcription and translation after sequencing the gene in a subset of Caucasians, African-Americans, Asians, and Hispanics.³⁸ Drysdale identified 13 polymorphisms and organized them into 12 haplotype groups with varying frequencies between ethnic groups (Table 1). The four most common haplotypes identified were haplotypes 1, 2, 4, and 6, representing Cys^{19(BUP)}/Arg¹⁶/Gln²⁷, Arg^{19(BUP)}/Gly¹⁶/Gln²⁷, Cys^{19(BUP)}/Arg¹⁶/Gln²⁷, and Cys^{19(BUP)}/Gly¹⁶/Gln²⁷, respectively (Table 1). HEK293 cells that were homozygotes for haplotype 2 exhibited a higher level of receptor expression and gene transcription compared to haplotype 4 homozygotes. The *in vitro* findings were consistent with *in vivo* findings of albuterol-induced FEV1 reversibility in 121 Caucasians: FEV1 reversibility was greatest in haplotype 2 homozygotes and lowest in haplotype 4 homozygotes.³⁸

The effect of the Thr¹⁶⁴Ile polymorphism on β2-adrenergic receptor binding affinity and coupling to Gs has been studied in CHW-1102 cells. Cells with the rare variant, Ile¹⁶⁴, exhibited decreased receptor binding affinity with epinephrine, isoproterenol, and norepinephrine. Furthermore, cells with the Ile¹⁶⁴ variant showed diminished receptor coupling to the Gs protein as determined by depression of adenylyl cyclase activity.⁴⁰

ADRβ2 Pharmacogenetics, Asthma Susceptibility, and Responsiveness to Acute SABA Therapy

In vitro studies suggest that two *ADRβ2* polymorphisms, Gly¹⁶Arg and Gln²⁷Glu, play a significant role in receptor down-regulation and desensitization and, in theory, may predict susceptibility to asthma phenotypes and the adverse effects of SABA therapy. These polymorphisms have served as the fundamental basis of interpreting *in vivo*, candidate gene analyses of *ADRβ2*. Several genetic association studies of *ADRβ2* have found Gly¹⁶Arg and Gln²⁷Glu genotypes to be associated with susceptibility to asthma and asthma-related phenotypes such as pulmonary function studies, bronchial hyperresponsiveness (BHR), and dependence on corticosteroid therapy, nocturnal symptoms, and atopy. However, later studies with similar or larger sample sizes and meta-analyses have not been able to replicate these findings, likely due to differences in subject race or ethnicity, insufficient sample size to detect a potentially weak genetic effect, and the exclusion of other *ADRβ2* polymorphisms from the analysis.^{34,42-57}

Despite the divergent results of *ADRβ2* genetic association studies, pharmacogenetic studies have been more consistent in describing the role *ADRβ2* polymorphisms in the pharmacological response to acute and regular SABA therapy. In 1997, Martinez and co-workers described one of the earliest pharmacogenetic relationships between Gly¹⁶Arg

genotypes and response to a single administration of albuterol in a group of 269 children participating in a longitudinal study of asthma.⁵⁸ In this study, Arg¹⁶ homozygotes and Gly¹⁶Arg heterozygotes were 5.3 times and 2.3 times more likely than Gly¹⁶ homozygotes to show a positive response to albuterol (FEV₁ increase greater 15.3% predicted), respectively. The study did not find a relationship between Gln²⁷Glu genotypes and albuterol response.⁵⁸ Another, study based on the NHLBI Childhood Asthma Management Program (CAMP) was a family-based genetic association analysis by Silverman and co-workers who analyzed the effect of eight ADRβ₂ polymorphisms on albuterol responsiveness in 707 asthmatic children.⁵⁶ In the CAMP cohort, Gly¹⁶Arg was significantly associated with post-bronchodilator FEV₁ with Arg¹⁶ homozygotes having higher FEV₁ percent of predicted values. In addition, a promoter polymorphism, Glu¹¹²Lys, and a noncoding SNP, Arg¹⁷⁵Arg, were also associated with post-bronchodilator FEV₁ and beta agonist responsiveness, respectively.⁵⁶ Several other smaller studies have observed similar effects of Arg¹⁶Gly genotypes on response to acute SABA therapy with the notable exception of a study by Taylor and co-workers.^{47, 58-63} Taylor and co-workers selected 176 patients based solely on a physician's diagnosis of asthma and found no relationship between Gly¹⁶Arg genotypes or ADRβ₂ haplotypes and responsiveness to a single dose of albuterol.⁶⁰

In the Genetics of Asthma in Latino Americans (GALA) study, Choudhry et al investigated the pharmacogenetic relationship of Gly¹⁶Arg genotypes with albuterol-induced FEV₁ reversibility in two ethnic groups (365 Puerto Rican asthmatics and 294 Mexican-Americans) through family-based and cross-sectional analyses.⁵⁹ In Puerto Ricans, the number of Arg¹⁶ alleles was associated with increasing FEV₁ reversibility and showed an additive effect that was more pronounced among those with a baseline FEV₁ less than 80% predicted. The association was not found in Mexican-Americans indicating a pharmacogenetic difference in the response to acute bronchodilator reversibility between these two ethnic groups.⁵⁹ Admixed ethnic groups such as African-Americans, Puerto Ricans, and Mexican-Americans have different ADRβ₂ polymorphism and haplotype frequencies.^{35,38,59} These genetic differences have the potential to cause ethnic group-specific pharmacogenetic differences in response to beta agonists, such as those observed by Choudhry and co-workers.⁵⁹

The haplotype-based findings of Drysdale indicate that albuterol-induced FEV₁ reversibility is greatest in haplotype 2 (Gly¹⁶) homozygotes and lowest in haplotype 4 (Arg¹⁶) homozygotes, an inconsistent observation based on other studies.³⁸ These conflicting findings may be better understood in the context of a haplotype effect from other adjacent ADRβ₂ genetic polymorphisms. In a haplotype, smaller genotypic effects of multiple, functional genetic polymorphisms, such as Arg¹⁹(BUP)Cys, may also determine receptor agonist response. Taylor and co-workers reported that the Drysdale haplotypes were not associated with albuterol-induced FEV₁ reversibility in 176 patients selected based on a physician's diagnosis of asthma, not airflow reversibility.⁶⁰ Similar findings were also observed by Hawkins³⁵. In contrast, Drysdale and coworkers selected patients based on a spirometric diagnosis of asthma selection criteria that may explain some of the inconsistent findings of these haplotype-based studies.³⁸ Airflow reversibility is primarily determined by baseline airflow obstruction (e.g., lower baseline FEV₁ values⁶⁴); therefore a requirement for bronchodilator reversibility selects for more subjects with lower baseline FEV₁ for analysis and tends to exclude those with milder disease.

To further elucidate the complexity of ADRβ₂ haplotypes and their effects on airflow obstruction, Hawkins and co-workers sequenced a 5.3kB region of ADRβ₂, including the promoter and untranslated regions, in 669 Caucasians and 240 African Americans identifying a total of 49 polymorphisms, which were used to construct extended, complex haplotypes (Figure 2). One of these polymorphisms includes a poly-cytosine repeat region of 10 to 14 cytosine repeats at nucleotide position +1069, 20 nucleotides adjacent to an AU-rich region

that determines mRNA stability (Figure 3). This region shows differences in length in different ethnic groups. (Figure 4) Poly-cytosine repeat lengths greater than 12 have been associated with baseline airflow obstruction in African Americans.³⁵ In a complex genetic disease such as asthma, it is possible that the smaller genetic effects of many polymorphisms throughout the *ADRβ2* gene and its untranslated regions may play a role in determining responses to beta agonist therapy.

ADRB2 Pharmacogenetics and the Response to Chronic, Regular SABA Therapy

Pharmacogenetic studies of regular SABA therapy have focused on the effects of Gly¹⁶Arg genotypes on beta agonist response, the most important area of research in asthma pharmacogenetics currently. Hancox and co-workers were among the first investigators to describe the genotypic effects of Gly¹⁶Arg during regular SABA therapy. This study was a retrospective genetic analysis of a placebo-controlled, cross-over trial comparing asthma control during regular versus as-needed fenoterol during a 24-week period. The analysis reported that Gly¹⁶ homozygotes using regular therapy experienced no increase in BHR while Gly¹⁶Arg heterozygotes experienced a significant increase in BHR during regular therapy.⁴⁵ Taylor and co-workers also analyzed the genotypic effects of Gly¹⁶Arg during regular LABA and SABA therapy using a retrospective analysis of a placebo-controlled, crossover trial of regularly scheduled albuterol and salmeterol therapy in 115 patients with asthma. Within the subset of Arg¹⁶ homozygotes, exacerbations occurred at a higher frequency during regular albuterol therapy compared to placebo but no adverse effects were noted during the use intermittent albuterol or during LABA (salmeterol) therapy. Furthermore, the Arg¹⁶ homozygotes experienced a decline in PEFr during albuterol therapy.⁶⁵ This finding was found in two different Asthma Clinical Research Network (ACRN) trials.⁶⁶⁻⁶⁷

The Beta-agonists study (BAGS) retrospective analysis of the ACRN BAGS trial evaluated the effects of Gly¹⁶Arg genotypes on daily peakflow during regular SABA therapy. In the BAGS trial, Isreal and co-workers retrospectively examined the effects of Gly¹⁶Arg genotypes in 190 patients with mild asthma treated with regular or as-needed receptor albuterol therapy over a 16-week period.⁶⁶ (Figure 5) At the end of the 16-week period, there was a decline in AM PEFr among Arg¹⁶ homozygotes using regular beta agonist therapy compared to those using as-needed therapy that was accentuated during the 4-week run-out period. (Figure 5) The evening PEFr also declined among Arg¹⁶ homozygotes on regular agonist therapy while there was no effect in the Gly¹⁶ homozygotes. Interestingly, Arg¹⁶ and Gly¹⁶ homozygotes on as-needed beta agonist therapy showed no effects on PEFr.⁶⁶

One of the first The BARGE trial was prospective pharmacogenetic studies (The BARGE Trial) was designed to examine the effects of Gly¹⁶Arg genotypes on asthma control during regular SABA therapy in asthma subjects selected and randomized based on individual genotypes.⁶⁷ Thus, the BARGE trial was a genotype-stratified, placebo-controlled, cross-over trial in which 37 Arg¹⁶ homozygotes and 41 Gly¹⁶ homozygotes with mild asthma were treated with regular or as-needed albuterol therapy over a 16-week period then crossed over to the alternative therapy. Additionally, rescue therapy with beta agonists was minimized by using ipratropium bromide as the primary reliever medication and albuterol being used only if ipratropium was found to be ineffective. Arg¹⁶ homozygotes showed an increased AM PEFr during treatment with placebo therapy, while Gly¹⁶ homozygotes had an increase in AM PEFr during regular albuterol therapy. (Figure 6) Furthermore, Arg¹⁶ homozygotes had significant adverse effects on FEV1, forced vital capacity (FVC), asthma symptom scores, and rescue inhaler use in comparison with regular albuterol therapy and placebo therapy. In contrast, Gly¹⁶ homozygotes showed significant improvements in forced vital capacity (FVC), asthma symptom scores, and rescue inhaler use during regular beta agonist therapy. Interestingly, during the run-in period,

an interval when albuterol use was minimized since ipratropium was used as the rescue medication, Arg¹⁶ homozygotes showed a significant increase in AM PEFR.⁶⁷

The contrasting genotypic effects of Gly¹⁶Arg genotypes on indices of asthma control (e.g., PEFR, BHR, exacerbations rates, etc.) during acute and chronic beta agonist therapy may have multiple explanations. One of these explanations takes into account both *in vivo* and *in vitro* observations: the so-called “dynamic model” of receptor kinetics. In this model, endogenous catecholamine activity results in receptor downregulation in Gly¹⁶ homozygotes, receptors known to have enhanced downregulatory activity *in vitro*. The tachyphylaxis observed during regular beta agonist therapy would, therefore, become more apparent in Arg¹⁶ homozygotes whose receptors are less likely to have downregulated prior to beta agonist therapy. Based on this model, the initial response to beta agonists among the beta-agonist-naïve would be lower in Gly¹⁶ homozygotes and be less likely to worsen over time. In contrast, Arg¹⁶ homozygotes would show a greater initial response to therapy but then undergo receptor downregulation during regular beta agonist therapy and, therefore, exhibit tachyphylaxis.⁶⁸

ADRB2 Pharmacogenetics and the Response to LABA Therapy

LABA's are effective anti-asthma medications that are indicated in the long-term management of chronic asthma in conjunction with inhaled corticosteroids. Pharmacogenetic studies have evaluated the genotypic effects of ADRβ2 polymorphisms on LABA response during chronic therapy. To determine whether genetic variants contribute to variation in response to LABA therapy. In the previously described retrospective analysis of a LABA and SABA crossover trial by Taylor and co-workers, Arg¹⁶ homozygotes experienced no adverse effects during LABA (salmeterol) in comparison to placebo (as needed SABA therapy) and regular SABA therapy.⁶⁵

A retrospective genetic analysis of two randomized, placebo-controlled salmeterol trials, the Salmeterol or Corticosteroids (SOCS) trial and Salmeterol +/-Inhaled Corticosteroid (SLIC) trial, was performed by the ACRN to determine a genotypic-difference in LABA response.⁶⁹ The SOCS trial was a 28-week trial comparing LABA monotherapy with inhaled corticosteroid (ICS) monotherapy in subjects with persistent asthma.⁷⁰ In contrast, the SLIC trial was a 24-week trial that examined the effects of adding LABA therapy to a stable or declining dose of ICS therapy.⁷¹ The SLIC trial concluded that persistent asthmatics who are optimally controlled by LABA and ICS combination therapy are able to have a 50% reduction in ICS dose without loss of asthma control; however, the elimination of ICS therapy results in a deterioration of asthma control.⁷¹

Ninety-six subjects from the SOCS trial and 74 subjects from the SLIC trial were genotyped based on eight ADRβ2 polymorphisms, including Gly¹⁶Arg.⁶⁹ Genotypic analysis of the SOCS and SLIC cohorts revealed that Arg¹⁶ homozygotes had a lower AM PEFR than Gly¹⁶ homozygotes during salmeterol therapy, particularly after week 10 of the trial. In SLIC, Arg¹⁶ homozygotes on LABA and ICS combination therapy also experienced a significantly lower FEV1, higher asthma symptom scores, and increased rescue inhaler use when compared to Gly¹⁶ homozygotes on similar therapy.⁶⁹ These results supported a ADRβ2 genotype effect on responses to therapy with the LABA's salmeterol.

In an attempt to further characterize the pharmacogenetic relationship between ADRβ2 polymorphisms and response to chronic LABA therapy in a larger sample size, Bleeker and co-workers genotyped five ADRβ2 SNP's in 183 subjects with persistent asthma who were randomized to regular salmeterol and ICS combination therapy or montelukast for a 12-week period followed by a 2 to 4 day run-out period. During the 12 week period, all subjects randomized to salmeterol therapy experienced sustained and significant improvements in AM PEFR regardless of Gly¹⁶Arg genotype. (Figure 7) During the run-out period, all subjects

exhibited a similar and predictable decline in asthma control.⁷² Issues with this clinical trial include the shorter 12-week duration of the study which may not have been long enough to detect the pharmacogenetic effects reported in the SOCS and SLIC trial. However, the findings reported by Bleecker and co-workers suggest that Gly¹⁶Arg genotypes may not be a genetic determinant of reduced responses to chronic LABA therapy in the presence of ICS therapy.⁷² More recently there have been two reports that do not show an effect of variation at the Arg¹⁶Gly locus on the response to LABA Therapy.^{73,74} In addition, the NHLBI ACRN network is currently investigating variation at the Arg¹⁶Gly locus in the Long Acting Beta Against response by genotype (LARGE) study.

Pharmacogenetics of LABA and SABA therapies: A Clinician's Perspective

The divergent effects observed in Arg¹⁶Gly genotype-specific responses to acute SABA therapy, chronic SABA therapy, and LABA therapy during most of these trials likely result from multiple issues. Important pharmacological areas include the presence of concomitant therapies such as ICS, the different intrinsic activities of the study agents (beta agonists), and the use of beta agonist rescue therapy in the majority of the trials. *In vitro*, corticosteroid therapy upregulates the β 2-adrenergic receptor, thereby, generating a drug-drug interaction with beta agonists. This interaction has the potential to mask the genotypic effects of ADR β 2 polymorphisms during concomitant LABA and ICS therapy. The use of other concomitant anti-asthma therapies may also have the potential of masking these genotypic effects through their clinical effects on asthma control. In addition, beta agonists with lower intrinsic activities (e.g. salmeterol) may be less likely to induce receptor downregulation and generate a detectable genetic effect at the clinical level.

A majority of pharmacogenetic studies to date are retrospective analyses of previous clinical trials; therefore, rescue bronchodilator therapy consisting of beta agonist therapy was an appropriate option. A problem is that the use of beta agonists as an as-needed, rescue therapy during a pharmacogenetic trial based on beta agonist response may effect genotype-specific responses to regular beta agonist therapy. As an example, it is possible that the observed beneficial effects of placebo among Arg¹⁶ homozygotes in the BARGE trial may have been attributed to the absence of beta agonist rescue therapy and its substitution with ipratropium rescue therapy. The substitution of beta agonist rescue therapy with ipratropium in the BARGE trial eliminates all beta agonist exposure during placebo therapy and alter receptor downregulation resulting in the observed benefit among Arg¹⁶ homozygotes in the BARGE trial. In contrast, the use of beta agonist rescue therapy throughout the BAGS trial possibly resulted in receptor downregulation.

Two common statistical limitations in the majority of these pharmacogenetic trials include insufficient sample sizes to detect the weak genetic effect of a single polymorphism and a limited number of polymorphisms reported in the genetic analysis. To date, most pharmacogenetic studies do not take into account the effects of other functionally relevant polymorphisms such as Arg¹⁹Cys or other polymorphisms in the 5' promoter and 3' untranslated region of ADR β 2 that may or may not be in linkage disequilibrium with the commonly analyzed Gly¹⁶Arg polymorphism.^{35,38} It is also possible that another polymorphism in linkage disequilibrium with Gly¹⁶Arg may be directly responsible for the observed genetic effects. Furthermore, since asthma is a complex genetic disease, individual polymorphisms likely exhibit a small genetic effect that may not be detectable in small sample size studies. This limitation is further complicated by the fact that ADR β 2 haplotype effects accounting for the combined effects of several polymorphisms are even more difficult to detect in small sample sizes.^{35,38} ADR β 2 polymorphisms may also interact with polymorphisms on other pathway-related genes, thereby causing gene-gene interactions that further modulate beta agonist responses.⁷⁵⁻⁷⁷ Other forms of genetic variation such as insertion-deletions, base-

repeats, and epigenetic changes have not been investigated as determinants of receptor downregulation or desensitization and beta agonist response.³⁵

Finally, there are differences in the study design of these pharmacogenetic studies that may account for their divergent results including differences in asthma severity of the study participants and a lack of recognition or representation from other ethnic or racial groups.^{35, 59} As discussed, criteria such as bronchodilator reversibility tends to select more severe patients with lower baseline FEV1 values, excluding those with milder asthma. Furthermore, studies such as BAGS and BARGE selected participants with mild asthma to minimize the use of concomitant medications, such as ICS, in the study design. In contrast, the study by Bleecker and co-workers and the retrospective analysis of SOCS and SLIC selected participants with persistent asthma and included concomitant ICS therapy in the study design. Variations in the genotypic effect of Gly¹⁶Arg observed in these studies could possibly be attributed to the differences in severity of the participants in each study or the use of concomitant ICS therapy.

Some of these studies fail to take into account individual ethnicity by either not stratifying study populations by ethnicity or excluding other ethnic or racial groups in the genetic analysis. Ethnic group-specific pharmacogenetic differences in response to beta agonist therapy, such as those observed between Mexican-Americans and Puerto Ricans, may be overlooked if ethnic groups are excluded from genetic analyses or if within a study population these ethnic groups are not stratified or analyzed separately.⁵⁶ Furthermore, failure to account for individual ethnicity or ancestry in a study population contributes to confounding by population stratification. Other important differences in study design between these pharmacogenetic trials include stratification by genotype with a prospective analysis versus stratification by drug-placebo status with a retrospective genotypic analysis, the inclusion of a cross-over design, varying duration of the studies, and the use of beta agonist or anti-cholinergic as rescue therapy.

Conclusions

The results of published studies clearly supports experimental evidence that variation in the ADRB2 gene at the Arg¹⁶Gly locus effects therapeutic responses during regular SABA therapy.^{45, 65-67} During regular therapy with LABA, reduced responses in Arg16 homozygotes have been reported in two small studies⁶⁹ but have not been found in several other larger studies.^{65, 72-74} It is unclear why there are differences in pharmacogenetic responses to short and long acting beta except that they may be related to the longer duration of action of LABAs. In view of the overall safety and efficacy of LABA therapy and the lack of a therapeutic benefit of regular SABA therapy, these pharmacogenetic observations should not alter the current standard of care which encourages the use of as-needed SABA therapy or regular LABA and ICS combination therapy to achieve optimal control of asthma symptoms.⁶ Understanding the effects of ADRβ2 gene and pathway variation on individual responses to beta agonist therapy will improve as prospective pharmacogenetic studies analyze the genetic effects of a larger number of ADRβ2 SNPs and haplotypes in larger, more ethnically diverse populations taking into account interactions with other genes and the environment.

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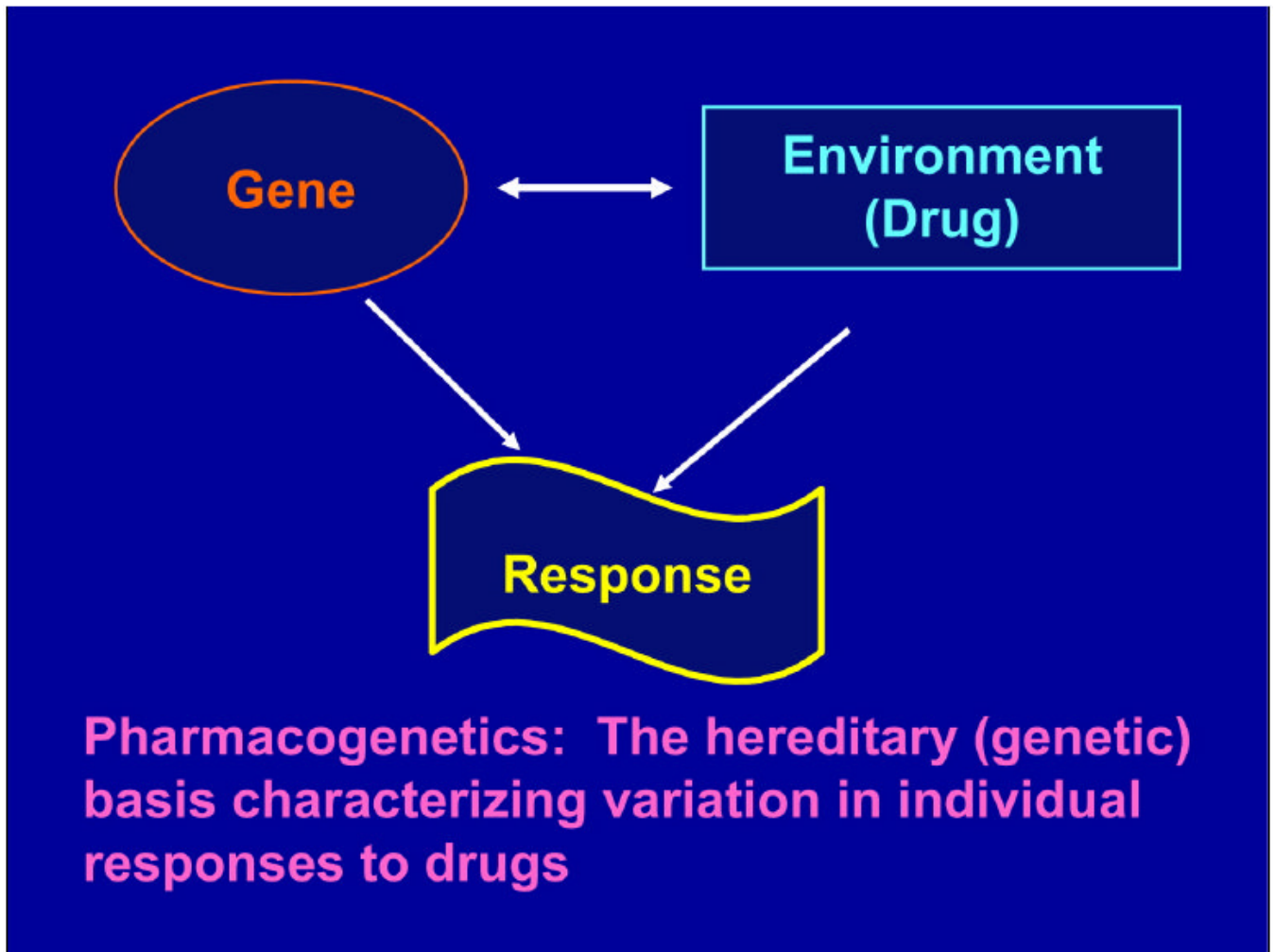


Figure 1.
No legend self explanatory

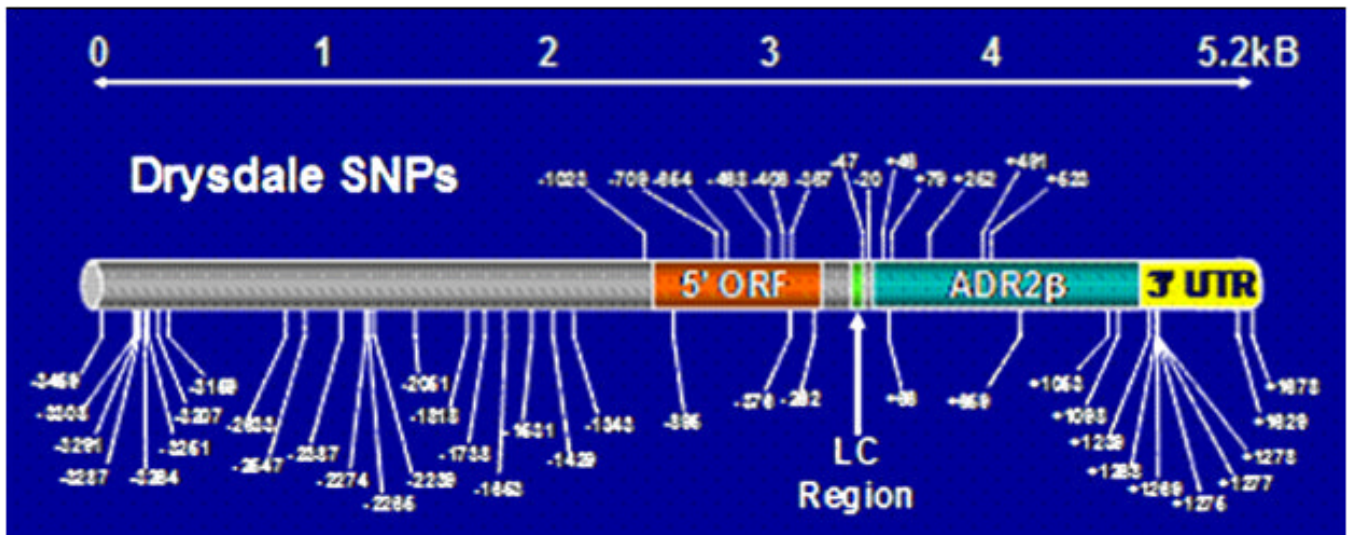


Figure 2.

Single Nucleotide Polymorphisms (SNPs) in the ADR β 2 gene. SNPs above the gene diagram were those reported by Drysdale et al³⁸ while more extensive resequencing of the gene by Hawkins et al³⁵ show significantly more variation in this one exon gene. (ORF=open reading frame, ADR β 2=exon region, LC=leader cistron, 3' UTR= 3' untranslated region)

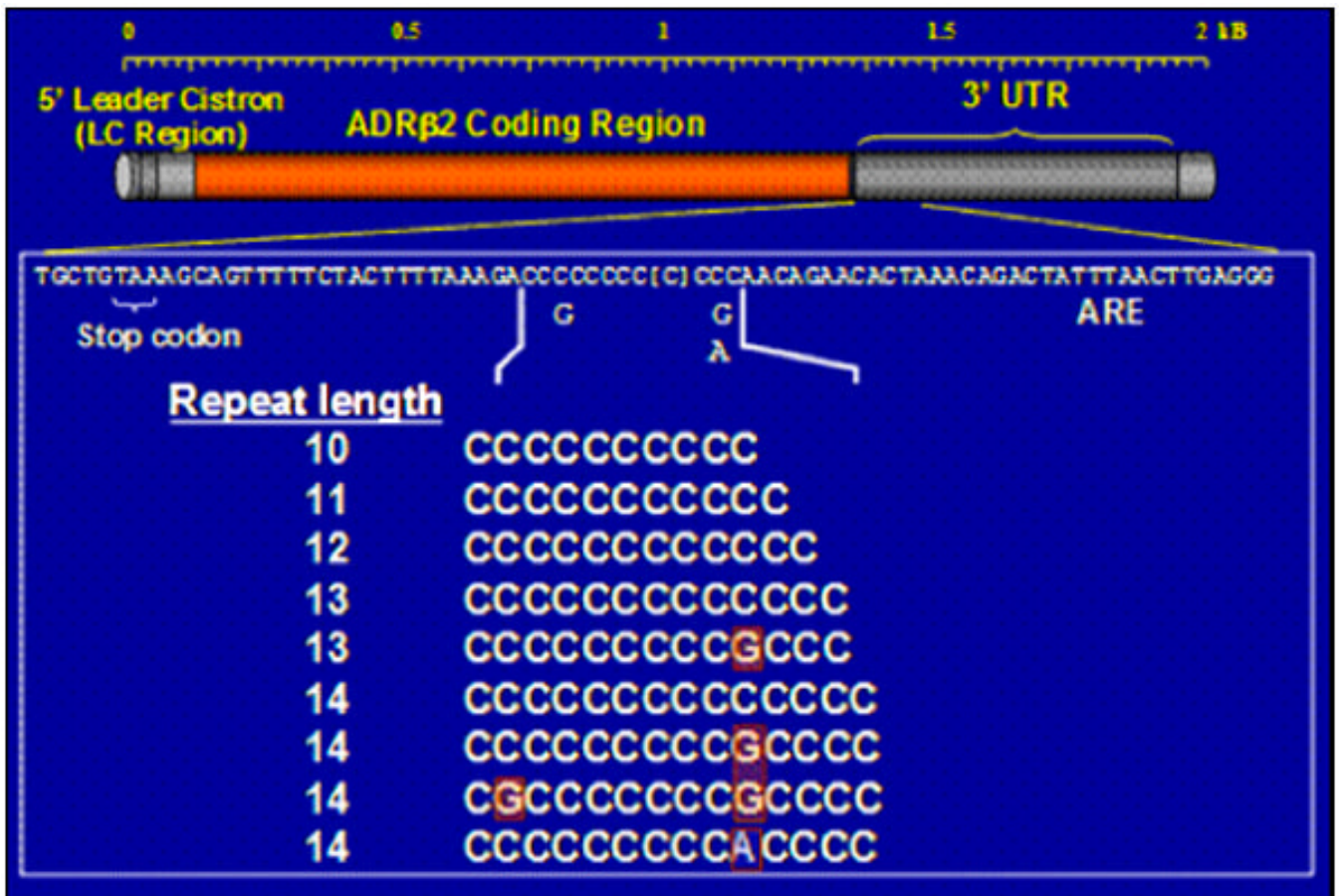


Figure 3. Illustrates poly C region with G/C variation in longer Poly C regions³⁵ (13,14 repeat lengths)

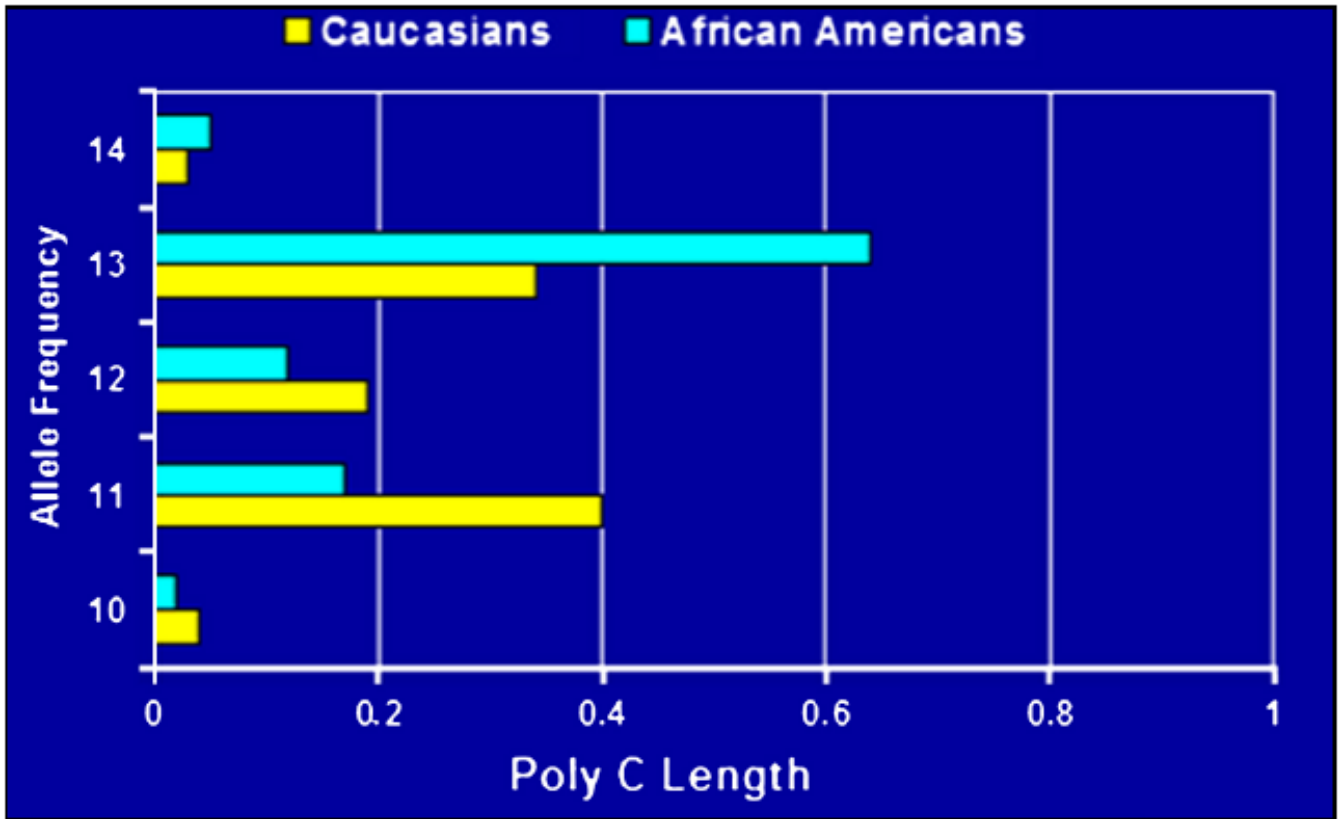


Figure 4. Length of Poly C region in Caucasian and African Americans³⁵

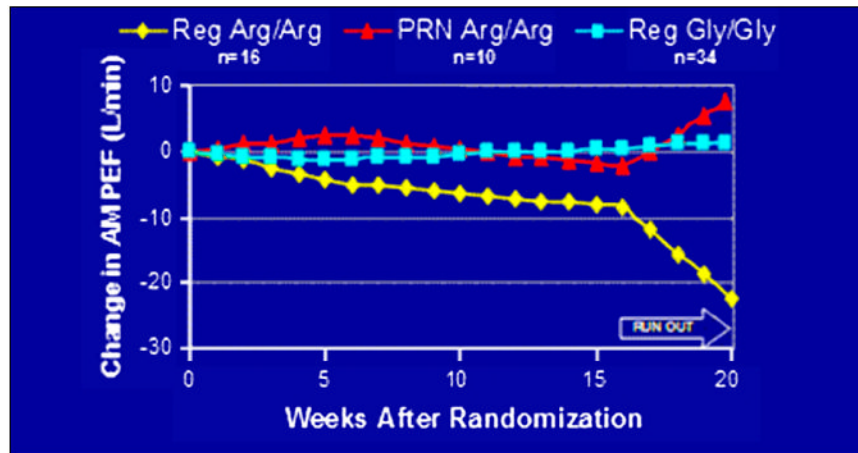


Figure 5. Responses to as needed albuterol therapy by genotype in the ACRN BAGS Study⁶⁶. Arg¹⁶ homozygotes on regular albuterol therapy showed a decrease in AM PEFR during the clinical trial and the run out period.

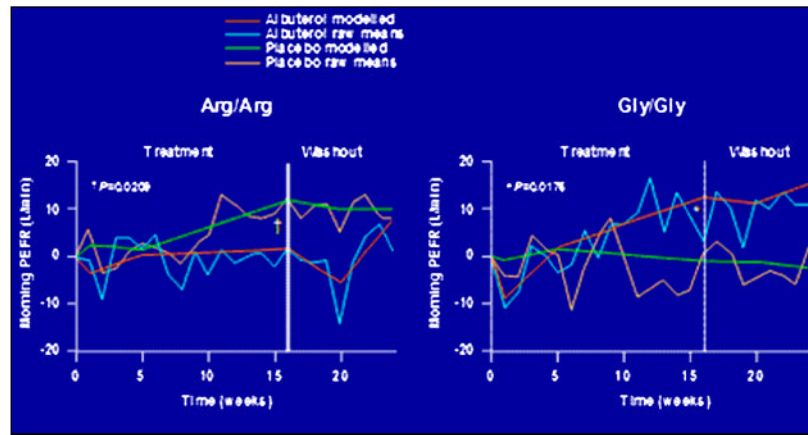


Figure 6. Responses to regular albuterol therapy and placebo (protoprium) in the ACRN BARGE study in Arg¹⁶ and Gly¹⁶ homozygotes⁶⁷.

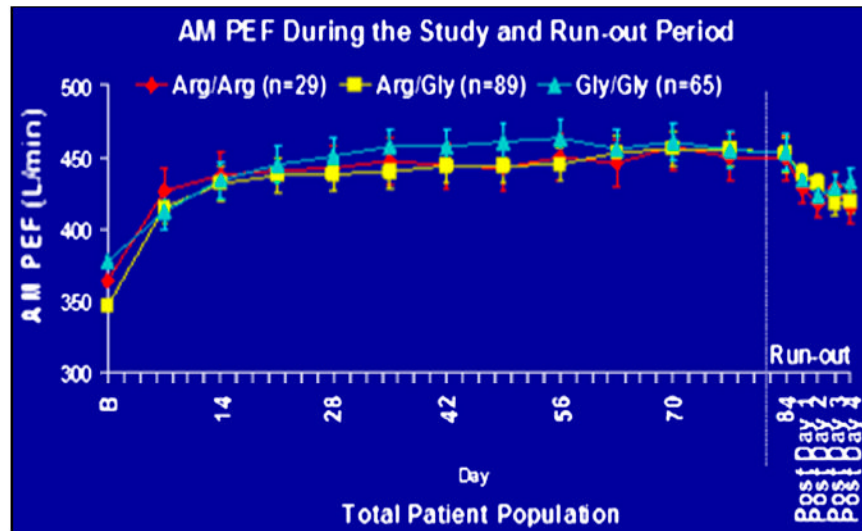


Figure 7. Responses to combination therapy with fluticasone and salmeterol in the Arg¹⁶ homozygotes. There was no difference in the response to therapy when stratified by variation of the Arg¹⁶Gly locus.⁷²

Table 1

Drysedale haplotypes and haplotype frequencies in different ethnic groups per Drysdale *et al* Hawkins *et al.**^{3,1,34}

Drysedale Haplotype Groups (5'UTR)	Polymorphism allele by nucleotide												Haplotype frequencies (%)						
	-1023 (5'UTR)	-709 (5'UTR)	-654 (5'UTR)	-468 (5'UTR)	-406 (5'UTR)	-367 (5'UTR)	-47 BUP (Cys/Arg19)	-20 (5'UTR) (Gly/Arg16)	+46 (Gln/Glu27)	+79 (Leu/Leu84)	+252 (Thr/Ile164)	+491 (Arg/Arg175)	+523 (Arg/Arg175)	CA per Hawkins <i>et al</i>	AA per Hawkins <i>et al</i>	CA per Drysdale <i>et al</i>	AA per Drysdale <i>et al</i>	HIS per Drysdale <i>et al</i>	AS per Drysdale <i>et al</i>
1	A	C	G	C	C	T	T	T	A	C	G	C	C	0	17	0.7	25	10	12.5
2	A	C	G	G	C	C	C	C	G	G	G	C	C	38	12	48.3	6.3	26.7	10
3	G	A	A	C	C	T	T	T	A	C	G	C	C	0	0	0.7	0	0	0
4	G	C	A	C	C	T	T	T	A	C	G	C	C	37	21	33	29.7	40	45
5	G	C	A	C	C	T	T	T	G	C	G	C	C	0	0	1.4	0	0	0
6	G	C	G	C	C	T	T	T	G	C	A	A	A	12	28	13.2	31.3	13.3	30
7	A	C	G	G	C	C	C	C	G	G	G	C	C	2	0	1	1.6	3.3	0
8	G	C	A	C	C	T	T	T	A	C	A	A	A	0	0	0.7	0	0	0
9	A	C	G	C	T	T	T	T	A	C	G	C	C	0	3	0	4.7	0	0
10	G	C	G	C	C	T	T	T	G	C	A	C	C	2	0	0.7	0	0	0
11	G	C	G	C	C	T	T	T	G	C	G	C	C	0	0	0.3	0	0	2.5
12	G	C	G	C	C	T	T	T	G	C	A	C	C	0	0	0	1.6	3.3	0

* 5' UTR=5' Untranslated Region, BUP=Beta Upstream Peptide.

^ CA=Caucasians, AA=African Americans, Hispanics=HIS, AS=Asians.