The Pharmacogenetics and Pharmacogenomics of Asthma Therapy

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Abstract

Despite the availability of several classes of asthma medications and their overall effectiveness, a significant portion of patients fail to respond to these therapeutic agents. Evidence suggests that genetic factors may partly mediate the heterogeneity in asthma treatment response. This review discusses important findings in asthma pharmacogenetics and pharmacogenomics studies conducted to date, examines limitations of these studies and finally, proposes future research directions in this field. The focus will be on the three major classes of asthma medications: β-adrenergic receptor agonists, inhaled corticosteroids and leukotriene modifiers. Although many studies are limited by small sample sizes and replication of the findings is needed, several candidate genes have been identified. High-throughput technologies is also allowing for large-scale genetic investigations. Thus, the future is promising for a personalized treatment of asthma, which will improve therapeutic outcomes, minimize side effects and lead to a more cost-effective care.

Keywords

asthma; pharmacogenetics; pharmacogenomics

Introduction

Asthma is a chronic inflammatory disease of the airways characterized by variable airflow obstruction and affects more than 300 million individuals worldwide. In the United States, the prevalence of asthma is 7.7% in adults and 9.6% in children, with similar figures in other developed countries ¹. A recent study using data from Medical Expenditure Panel Survey (MEPS) estimated the annual total cost of asthma in the USA, which includes incremental direct costs and productivity costs, to be $56 billion in 2007 ². Prescription medications accounted for the largest direct expenditure for asthma. Despite the wide availability of therapeutic asthma drugs and large studies supporting their efficacy, there is significant interindividual variability in the response to each of the three major classes of asthma medications ³. An efficient method to differentiate non-responders from responders prior to initiating treatment would not only avoid unwanted adverse effects, but also significantly decrease the economic burden of this disease.

Asthma has been long recognized as a genetic disease through observation of clustering of disease within families and twin studies. Population-based twin studies suggest that...

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Conflicts of interest

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heritability estimates for asthma range from 53% up to 92% \(^4\). Nonetheless, asthma is a complex genetic disease that is likely the result of multiple gene-gene and gene-environment interactions. The environmental risk factors associated with incident asthma include smoke exposure, certain viral infections, allergen exposures and vitamin D deficiency.

The following sections highlight a selection of important as well as recent studies in the pharmacogenetics and pharmacogenomics of asthma. Each of the three major therapeutic drug classes will be discussed, which includes \(\beta_2\)-adrenergic agonists, corticosteroids, and leukotriene modifiers.

**Harmonization of asthma treatment response phenotypes**

In pharmacogenetic studies, response to asthma therapy has been frequently assessed using quantitatie methods, such as change in FEV\(_1\) (forced expiratory volume in 1 second), PC\(_{20}\) (provocative concentration of methacholine causing a 20% drop in FEV\(_1\), which reflects the degree of airway hyperresponsiveness, and bronchodilator response (change in FEV\(_1\) shortly after administration of a short-acting \(\beta_2\)-agonist). These measures of lung function are reproducible and their quantitative nature allows for increased statistical power in genetic analyses. Peak expiratory flow rate (PEFR) has also been used as a response phenotype, although it correlates poorly with symptoms and may lack reproducibility \(^5\,^6\). Although qualitative measures, such as asthma exacerbations or presence/absence of symptoms, do not fully correlate with measures of lung function \(^7\,^9\), they may nonetheless be useful pharmacogenetic outcome measures. In general, the change in lung function associated with asthma treatment administration follows a near-normal distribution, demonstrating substantial interindividual variability \(^10\,^11\), with a significant proportion of both non-responders and high responders to therapy. This wide variability in interindividual response, combined with high intraindividual repeatability, suggests a genetic basis to the heterogeneity in asthma treatment response \(^3\).

Harmonization of the treatment outcome phenotypes is essential for comparison between pharmacogenetic studies for several reasons, including the assurance that a given pharmacogenetic locus, if valid, will be generalizable. Moreover, different study populations are often combined to increase the statistical power to detect associations. We have previously demonstrated that, despite widely variant entrant criteria, the lung function response to inhaled corticosteroids both follows a relatively normal distribution and is remarkably similar across multiple clinical trial populations \(^12\). While the acute bronchodilator response distribution is slightly skewed, it too demonstrates remarkable reproducibility across populations. For instance, the SNP Health Association Resource (SHARE) Asthma Resource project (SHARP) (www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000166.v1.p1) is conducting genome-wide analyses of adults and children who have participated in National Heart, Lung, and Blood Institute’s clinical research trials on asthma. In the evaluation of acute bronchodilator response across the three major asthma networks contributing to SHARP, heterogeneity of response within each group of network subjects as well as similarity of response distribution between groups are noted (Figure 1). Together, the data from both the inhaled corticosteroid and bronchodilator response distributions indicates that there are factors, such as genetics, independent of the drug itself that determine relative degree of response to asthma medications and that these factors are common across a wide range of subjects. These findings suggest that there is a consistent pharmacogenetic effect across populations independent of asthma severity. In the context of genome-wide association analyses of asthma treatment response, this supports the combination of different clinical trials for more powerful analyses, as well as the ability to successfully independently replicate pharmacogenetic loci.
The β2-Adrenergic Receptor Agonists Pathway

β2-adrenergic receptor agonists are the most commonly prescribed medications in the treatment of asthma. Short-acting β2-agonists (SABAs) are the mainstay of treatment for acute symptoms of bronchospasm while long-acting β2-agonists (LABAs) are used as adjuncts to inhaled corticosteroid therapy to provide long-term asthma control. Both SABAs and LABAs exert their therapeutic effects via their binding to the active site of β2-adrenergic receptors (β2-AR), which are densely located on the smooth muscle cells of the lower respiratory tract. When stimulated, the β2-AR couples to adenylate cyclase through a trimeric G-protein, leading to increased production of cyclic adenosine monophosphate (cAMP) and protein kinase A (PKA). PKA phosphorylates key regulatory proteins involved in the control of muscle tone while cAMP results in sequestration of intracellular Ca++, both causing relaxation of the airway smooth muscle.

Short-acting β2-agonists (SABA)

A substantial fraction of the interindividual variability in response to SABA treatment is attributed to genetic factors. Many studies have examined the pharmacogenomics and pharmacogenetics of β2-agonists. However, as a whole, they have proven to be inconclusive due to the wide variability in study design and their definition of therapeutic end points.

The literature on β2-agonist treatment heterogeneity in asthma is dominated by studies on the ADRB2 gene, which encodes the β2-AR. This gene is a small, intron-less gene located on chromosome 5q31 and has been resequenced in several populations. Most studies have focused on Arg16Gly and Glu27Gln, two common nonsynonymous single nucleotide polymorphisms (SNP) at positions +46 and +79 on ADRB2 respectively. The reported minor allele frequency (MAF) for Arg16Gly (Gly16 being the minor allele) is around 40% for whites and African-Americans.

Martinez et al genotyped 269 children of Hispanic or Caucasian descent, 78 (29.2%) of whom reported episodes of wheezing during the previous year. They found that children who were homozygous for Arg16 were 5.3 times more likely to respond to a single dose of albuterol than the children who were homozygous for Gly16. There was a non-significant trend for association between Glu27Gln and bronchodilator responsiveness. Similarly, in a family-based study of Latino Americans with asthma, a highly significant association was found between the number of Arg16 alleles and responsiveness to a single dose of albuterol among asthmatic Puerto Ricans with a baseline FEV1 <80% predicted. This relationship was not present in Mexican asthmatics. Along with the finding of different linkage disequilibrium patterns between ADRB2 SNPs in Puerto Ricans and Mexicans, this study underscores ethnic-specific pharmacogenetic differences in the effect of Arg16Gly genotype on drug response.

The relationship between the Arg16Gly genotype and long-term use of SABAs has also been examined. Israel et al genotyped the Arg16Gly and Glu27Gln SNPs in 190 subjects who were randomized to regular plus as needed albuterol or as needed albuterol. When stratified by genotype, regular β2-agonist use was associated with a decline in morning and evening peak expiratory flow rates (PEFR) in patients who were homozygous for Arg16, but not other genotypes. While this genotype-specific effect was noted during the 16-week study period, the effect was greatest during the run-out period, during which all patients returned to using as-needed albuterol. The authors postulated that Arg/Arg patients could be more susceptible to the tachyphylactic effect of regular β2-agonist use as previous work has shown that β2-ARs are more downregulated at baseline in Gly/Gly patients compared to Arg/Arg patients. The higher concentration of β2-ARs at baseline in Arg/Arg patients would also be consistent with the findings above that Arg/Arg patients have a greater response to SABAs.
bronchodilator response to single dose albuterol. These results led to the first genotype-stratified, prospective placebo-controlled trial of asthma therapy. The BARGE trial (Beta-Adrenergic Response by Genotype) randomized 78 subjects to either regular albuterol or placebo for 16 weeks, followed by a crossover in treatment for another 16 weeks. Arg/Arg subjects had lower PEFRs during treatment with regular albuterol compared with placebo (−10L/min, range −19 to −2). Although this is statistically significant, the small difference may not be clinically significant. Since ipratropium bromide was used as rescue medication throughout the study, it was hypothesized that even as-needed use of SABAs may be unsafe for Arg/Arg patients.

**Long-acting β2-agonists (LABA)**

The concern for safety of β2-agonists was also extended to long-acting β2-agonists. Wechsler et al examined multiple SNPs on ADRB2 in a sample of subjects previously enrolled in clinical trials involving the LABA salmeterol. They found that Arg/Arg patients had a diminished therapeutic response to the regular use of salmeterol compared to those with Gly/Gly, as evidenced by a decrease in PEFR. However, this study’s impact was limited by its small sample size. Bleecker et al performed a similar analysis but found contradictory results, with no evidence of association between the Arg16Gly genotype and response to salmeterol in combination with ICS. This led to two larger, genotype-stratified, randomized controlled trials. In the LARGE study (Long-Acting Beta Agonist Response by Genotype), a total of 87 patients were randomized to 18 weeks of treatment with salmeterol or placebo with a subsequent crossover. Open label use of beclomethasone and ipratropium bromide was allowed throughout the trial. Both Arg/Arg and Gly/Gly patients showed improved PEFRs after treatment with salmeterol. Interestingly, Gly/Gly patients had enhanced bronchoprotection after treatment with salmeterol and inhaled corticosteroids, as evidenced by a higher PC_{20}, while there was no such difference in Arg/Arg patients. Recently, Bleecker et al published a large genotype-stratified clinical trial where 544 asthmatic subjects with Arg/Arg, Gly/Gly or Arg/Gly at codon 16 were randomized to salmeterol alone or in combination with inhaled fluticasone propionate for 16 weeks. Both Arg/Arg and Gly/Gly subjects showed improvement in morning PEFR with salmeterol alone and salmeterol in combination with fluticasone propionate, although the differences in PEFR were not statistically significant. Furthermore, Arg16Gly genotypes did not modify other secondary treatment responses, including evening PEFR, FEV_{1}, as needed ipratropium bromide use and percent of symptom-free days. The authors conclude that there was no evidence of a pharmacogenetic effect of ADRB2 gene polymorphisms on salmeterol response, in particular at the Arg16Gly position. Although the number of black subjects was small and unbalanced by genotype (n=130, with more Arg/Arg compared Arg/Gly and Gly/Gly), post hoc analyses in this subgroup showed that there was also no differential response to salmeterol based on the subjects’ Arg16Gly genotype. These results corroborate with previous publications showing no correlation between Arg16Gly and adverse outcomes associated with LABA use in the black population. Such findings are particularly relevant in the context of the previous SMART trial (Salmeterol Multicenter Asthma Research Trial), a randomized controlled trial comparing the safety of salmeterol or placebo added to usual asthma care. Interim analyses showed that significantly more African-Americans on salmeterol experienced respiratory-related death or life-threatening experience compared with those on placebo (RR 4.1 (1.5 to 10.9)). Due to these preliminary findings in African-Americans and difficulties in enrollment, the trial was stopped prematurely. Overall, while the combined findings from the LABA studies may be reassuring, the use of a LABA monotherapy remains contraindicated in the treatment of asthma because of the increased risk of severe exacerbation of asthma symptoms in some patients.
The majority of the pharmacogenetic studies on β2-agonist response have focused on the Arg16Gly polymorphism. However, the conflicting results from previous studies and the lack of evidence of clinical significance associated with this polymorphism so far suggest that there are likely other variants in partial linkage disequilibrium with Arg16Gly. Drysdale et al organized 13 SNPs from the ADRB2 gene into 12 haplotypes with highly variable frequencies across different ethnic populations. Individuals homozygous for the most common haplotype had an acute bronchodilator response that was twice as high as individuals homozygous for the second most common haplotype. In contrast, there was no association between individual SNPs and bronchodilator response, suggesting that haplotype analysis is more powerful in detecting associations than single SNP analysis. More recently, Hawkins et al comprehensively sequenced a 5.3-kb region of ADRB2 in 429 white and 240 African American subjects. They identified 20 previously unreported SNPs found in regulatory regions that contribute to haplotypic structure and to genetic association with asthma-related phenotypes. These studies support the need for comprehensive analysis of the ADRB2 gene, including haplotype analysis, in future pharmacogenetic studies. Animal studies have noted the importance of the 3′UTR to ADRB2 gene function and studies of regulatory variation in the gene would also be of interest.

In addition to ADRB2, a number of other genes from the β2-agonist pathway have been examined for pharmacogenetic effects. These include S-nitrosoglutathione reductase (GSNOR), which metabolizes the endogenous bronchodilator S-nitrosothiol (SNO). Increased GSNOR expression and decreased SNO levels have been demonstrated in the bronchoalveolar fluid of adults with mild asthma. Furthermore, GSNOR expression in bronchoalveolar lavage cell lysates was positively correlated with airway hyperreactivity. Choudry et al recently sequenced the GSNOR gene in a subset of a Mexican and Puerto Rican asthmatics cohort and identified 13 SNPs with an allele frequency greater than 5%. Association studies between 5 GSNOR SNPs (selected based on location and LD pattern) identified gene-gene interactions between GSNOR and ADRB2 in Mexicans and Puerto Ricans combined, although these SNPs and their haplotypes were not associated with bronchodilator response after Bonferroni correction for multiple testing. Specifically, asthmatic patients with the GSNOR+17059 TG+GG genotypes and either the ADRB2+46 AG or GG genotypes had a decreased bronchodilator response, with a mean change in FEV1 of 5.29% (AG) and 0.54% (GG) predicted, compared to 10.04% for the other genotypes.

Several recent studies support the role of ARG1 polymorphisms in bronchodilator response. Litonjua et al used a novel family-based screening algorithm based on each SNP’s statistical power to rank 844 SNPs in 11 bronchodilator response candidate genes in a cohort of 209 children and their parents participating in the Childhood Asthma Management Program (CAMP). Genes that had SNPs with median power in the highest quartile were subsequently taken for replication analyses in three other asthma cohorts. Four SNPs from ARG1 showed the strongest evidence for association with bronchodilator response, with 1 SNP (rs2781659) surviving Bonferroni correction. Three of the 4 SNPs were in strong linkage disequilibrium.

ARG1 encodes arginase, which controls L-arginine homeostasis. The availability of L-arginine to nitric oxide synthase (NOS) determines the production of nitric oxide, a bronchodilator. Increased ARG1 expression has been shown in human asthma bronchoalveolar epithelial and inflammatory cells, supporting the regulatory role of ARG1 in airway function. Further supporting the role of ARG in bronchodilator response, Vonk et al identified an association between the ARG1 SNP rs2781667, ARG2 SNPs rs7140310 and rs10483801 and β2-agonist response in 200 adults with a history of asthma. This ARG1 SNP was in high linkage disequilibrium with the SNP reported by Litonjua et al, thus replicating the previous findings. ARG1 was recently resequenced in a population of non-
Hispanic Whites, African-Americans and Hispanics to comprehensively identify genetic variants and the most prevalent haplotypes. In addition, the authors performed a bronchodilator response association study in 3 adult asthma populations. Using the 22 polymorphisms identified, 14 of which were located in the 5′ region of the ARG1 gene, 3 haplotypes with frequencies >5% were constructed, representing 80–91% of the 3 asthma populations. Individuals with the most prevalent haplotype (haplotype 1) were up to twice as responsive to albuterol as the individuals with the least prevalent of the 3 haplotypes (haplotype 3). In addition, human airway epithelial cells transfected with haplotype 1 showed greater reporter activity than the 2 other haplotypes, suggesting that ARG1 haplotypes regulate gene expression in vitro. The evidence supports a significant role for ARG1 variants in bronchodilator response. However, larger prospective genotype-stratified studies are needed to better understand arginase regulation and the effect on asthma treatment response.

Several candidate genes have been identified for the response to bronchodilators. However, few studies have attempted to integrate the existing genetic data into a predictive model. Recently, Himes et al modeled bronchodilator response in the CAMP cohort using Bayesian networks, which are multivariate models that are able to account for several interactions and associations among variables simultaneously. The bronchodilator response Bayesian network, constructed from 15 SNPs in 15 candidate genes, was better at predicting bronchodilator response than single SNPs. The integration of genetic data will be essential in the creation of accurate prediction models in pharmacogenetics, and multivariate models such as Bayesian networks are an attractive approach.

In summary, studies have identified several genes that may influence the response to short-acting and long-acting β2-agonists. This pharmacogenetic response also varies by ethnic group. Most of the literature has focused on the ADRB2 gene, especially with regards to the safety of LABAs, a widely prescribed class of asthma medication. Recent evidence suggests that the extensively studied Arg16Gly polymorphism does not play a significant role in the response to LABAs and is not associated with adverse effects. Future studies should consider the addition of other variants such as those in GSNOR and ARG1 in prediction models and perform association studies using haplotypes rather than individual SNPs. Genome-wide association studies for bronchodilator response are also under way, which may help improve the genetic prediction of bronchodilator responsiveness.

The Corticosteroid Pathway

Inhaled corticosteroids (ICSs) are considered first-line therapy and are the most effective anti-inflammatory drugs for the treatment of persistent asthma. Glucocorticoids (GC) exert their therapeutic effects by binding and forming a complex with the intracellular GC receptor. This complex is activated and translocates into the nucleus to directly alter transcription of genes involved in inflammation through binding with DNA or indirectly through interaction with other transcription factors. For example, GC inhibits gene expression of IL-3, IL-5 and IL-6, which are pro-inflammatory mediators involved in inflammatory cell proliferation and eosinophil regulation, while enhancing the expression of IL-10 and IL-12, whose roles include suppression of macrophage activation and differentiation of T cells to the Th1 subtype. In vitro studies have also shown that glucocorticoids can prevent and reverse the desensitization caused by chronic exposure to a β2-agonist. In the case of LABA’s, this may be mediated by the GC-induced upregulation of mitogen-activated protein kinase phosphatase 1, which leads to decreased levels of the pro-inflammatory phosphodiesterase.
Although there is significant interindividual variability in ICS responsiveness\(^3,4\), the intraindividual response is highly repeatable, supporting a genetic basis for the heterogeneity of ICS response\(^3\). A study of 14 candidate genes in the corticosteroid pathway revealed a significant association between corticotropin-releasing hormone receptor 1 (\(CRHR1\)) genotype and 8-week response to inhaled corticosteroid in two independent populations of adult and pediatric asthmatics\(^12\). Specifically, patients homozygous for the minor allele of rs242941 (MAF of 30\%) had a significantly higher change in percent FEV\(_1\) after ICS treatment, resulting in an average 2–3 times improvement compared to those without the variant. SNPs in CRHR1 were also organized into 4 haplotypes, distinguished by 3 haplotype-tag SNPs, rs1876828, rs242939 and rs242941. Homozygosity for the GAT haplotype (frequency 27\%) was associated with greater improvement in FEV\(_1\) after ICS treatment. Corticotropin-releasing hormone (CRH) is an important mediator of the endocrine, autonomic and immune systems’ response to stress. It binds to \(CRHR1\) in the pituitary gland and modulates the release of adrenocorticotropic hormone-induced cortisol production. Peripherally, CRH may exert a pro-inflammatory effect through mast cell degranulation\(^49\) and alterations in the CRH pathway have the potential to influence the pathogenesis of asthma. In fact, \(CRH\)-knockout mice subjected to ovalbumin-induced airway inflammation showed evidence of lung mechanical dysfunction, increase airway inflammation and goblet cell hyperplasia\(^50\). In contrast to Tantisira’s study, Dijkstra et al studied the 3 haplotype-tag SNPs in CRHR1 and found no association between CRHR1 genotype and long-term lung function decrease in adults, independent of treatment with ICS\(^51\), suggesting that there are other factors responsible for the ICS response variability. Recently, our group demonstrated that the association between CRHR1 and lung function response to ICS may be mediated by a large structural inversion on chromosome 17, with which \(CRHR1\) is in strong linkage disequilibrium\(^52\).

An obvious candidate to explain ICS response variability is the glucocorticoid receptor (GR). GR is part of a large heterocomplex of proteins and variations in genes encoding any of these components might contribute to variable interindividual steroid response. Hawkins et al. recently genotyped SNPs in 8 glucocorticoid-complex genes in 382 white subjects on inhaled corticosteroids and found that several SNPs in the \(STIP1\) gene were associated with percent change in FEV\(_1\) after 4 and 8 weeks of treatment\(^53\). Rs6591838 was associated with the largest increase in FEV\(_1\) (20.70\% ± 28.29\%) after 8 weeks, with the rare GG allele. Haplotype analysis also showed promising positive correlations between haplotypes and percent change in FEV\(_1\). However, replication of these findings is needed to confirm these observations.

\(TBX21\), another candidate gene, encodes for the nuclear transcription factor T-bet. T-bet is responsible for the differentiation of naïve T lymphocytes into Th1 cells while simultaneously repressing Th2 cells\(^54\). Compared to controls, asthmatic subjects have significantly decreased expression of T-bet expression in their peribronchial CD4+ lymphocytes\(^55\). Rs2240017, a nonsynonymous variation coding for replacement of histidine 33 with glutamine (H33Q, MAF 4.5\% in Caucasians), has been associated with marked improvement in airway hyperresponsiveness in children on ICS therapy\(^56\). On the other hand, subjects on ICS who were homozygous for histidine (H33H) demonstrated only a slight improvement in PC\(_{20}\) compared to the placebo group. Resequencing of the T-bet locus identified 7 haplotypes from 24 SNPs, several of which were associated with airway hyperresponsiveness\(^57\).

\(FCER2\) encodes for CD23, a low affinity IgE receptor and glucocorticoids have been shown to decrease \(FCER2\) expression and CD23 production\(^58\). However, a variant in \(FCER2\), T2206C, has been recently associated with increased IgE levels, severe asthma exacerbations in asthmatic children despite ICS use\(^59\). This SNP was not infrequent in
white subjects, with a MAF of 26%. White children homozygous for the T2206C mutant allele were 3.95 times (95% CI 1.64–9.51) more likely to have a severe exacerbation compared to all other T2206C genotypes. African American children demonstrated a similar trend with 3.08 (95% CI 1.00–9.47) increased risk of exacerbations for those homozygous for the mutant allele. Of further interest, in both White and African American children, the variant demonstrated no effect on the risk of exacerbation in those children not taking inhaled corticosteroid therapy. Incorporating this variant into a prognostic model may identify those subjects at risk of severe exacerbations and who may benefit from therapies other than ICS.

Due to issues with population stratification, most studies in ICS pharmacogenetics have examined associations in white subjects. A recent study looked at the association between SNPs in the DUSP1 gene (Dual-specificity phosphatase 1) and effect modification by ICS on bronchodilator responsiveness in Latinos (GALA study). The findings were subsequently replicated in African-Americans (SAGE and SAPPHIRE studies). DUSP1 is also known as mitogen-activated protein kinase (MAPK) phosphatase 1 (MKP1) and is induced by corticosteroids, resulting in inhibition of MAPK and hence the production of pro-inflammatory cytokines. In the above study, rs881152 in DUSP1 (GG genotype) was associated with greater bronchodilator response when ICS was used concurrently among the GALA and SAPPHIRE populations. Among patients who were treated with ICS for 6 weeks prospectively (SAPPHIRE), the GG genotype at rs881152 was also associated with improved self-reported asthma control compared to AG or AA genotypes.

Genomics studies have also begun to focus on differential expression of genes associated with ICS treatment response. For example, corticosteroid treatment down-regulated expression of CLCA1, periostin and serpinB2 while up-regulating FKBP51 expression in airway epithelial cells from asthmatic patients. High baseline expression of CLCA1, periostin and serpinB2 was correlated with improvement in FEV1 after 4 weeks of ICS treatment, whereas high expression of FKBP51 was associated with a poor response. Another study looking at genome-wide gene expression profiles in peripheral blood mononuclear cells derived a prediction model comprised of 11 genes that could discriminate glucocorticoid-sensitive and glucocorticoid-resistant asthmatic patients with 84% accuracy. Among these 11 genes, the NFKB gene alone predicted glucocorticoid resistance with 81.25% accuracy. The findings from these studies require replication. Nonetheless, they identified novel candidates for further investigation.

The breadth of research on the genetics and genomics of ICS response is less extensive compared to the studies in bronchodilator response. Several candidate genes and their functional variants have been identified, and although promising, most results need replication in future studies.

The Leukotriene Antagonist Pathway

Leukotrienes play a central role in a variety of inflammatory diseases such as asthma. In particular, increased levels of urinary leukotriene E4 (LTE4) have been associated with aspirin-exacerbated respiratory disease and exercise-induced bronchoconstriction. The leukotriene pathway begins with the conversion of arachidonic acid to leukotriene A4 (LTA4), a reaction catalyzed by the enzyme 5-lipoxygenase (5-LO). LTA4 is subsequently converted to leukotriene C4 under the influence of leukotriene C4 synthase (LTC4S), which is transported extracellularly. Sequential cleavage of glutamate and glycine residues results in the formation of leukotriene E4 and D4. Leukotrienes bind to receptors present on leukocytes and lung smooth muscle cells, such cysteinyl leukotriene receptors 1 (cysLTR1), to cause smooth muscle contraction and mucus secretion. Current anti-
leukotriene therapies include cysteinyl leukotriene receptor blockers (e.g. montelukast, zafirlukast, and pranlukast) and inhibitors of 5-LO (e.g. zileuton). However, the response to treatment with leukotriene modifiers among asthmatics is highly heterogeneous.  

ALOX5, the gene coding for 5-LO, has a tandem repeat polymorphism (factor Sp1-binding motif) within its promoter region that has been associated with diminished promoter-reporter activity. In a clinical drug trial of a 5-LO inhibitor, ABT-761, there was an improvement of 19–23% in FEV1 at the end of the treatment period for asthmatics who were homozygous or heterozygous for the wild type allele (homozygous or heterozygous for 5 repeats), while no change in FEV1 was observed those homozygous for mutant alleles (combinations of 3, 4, or 6 repeats). These findings were supported by a more recent study by Telleria et al that reported decreased number of asthma exacerbations, improvement of FEV1 and decreased use of β2-agonists following treatment with montelukast in asthmatics with at least one wild-type allele.

Klotsman et al investigated 25 polymorphisms in 10 leukotriene pathway candidate genes in a group of asthmatics randomized to montelukast for 12 weeks. Analyses were performed on ethnically diverse subjects, with 79% Caucasians, 22% Hispanics and 10 African-Americans. Variants in CYSLTR2 (rs912277 and rs912278) and ALOX5 (rs4986832) were associated with an 18–25% improvement in PEFRs, in contrast to 8–10% in subjects with the wild-type alleles. Lima et al genotyped 28 SNPs from genes involved in the leukotriene pathway and performed association studies on 61 subjects taking montelukast as part of a larger clinical study. In contrast to Klotsman’s study, this analysis was restricted to white subjects. Two outcomes were analyzed: change FEV1 after 6 months of montelukast therapy and presence of asthma exacerbations. Two SNPs were associated with change in FEV1, one in the ALOX5 gene (rs2115819), which was later replicated in a zileuton treatment response study, and one in the MRPI gene (rs119774). MRPI (multidrug resistance protein 1) transports LTC4 into the extracellular space and is highly expressed in human bronchial epithelial cells. A variation in LTA4H (rs2660845) was associated with a four-fold increase in the risk of asthma exacerbation over the treatment period, whereas LTC4S rs730012 and the mutant ALOX5 repeat polymorphisms were associated with over 70% reductions in exacerbations. This last finding seems to contradict the results from the Telleria study, which may be explained by the small sample sizes of the studies. However, taken together, these data suggest that the ALOX5 repeat polymorphism is an important pharmacogenetic locus.

Another polymorphism in the leukotriene pathway associated with response to leukotriene modifiers is the LTC4S C-444A locus. Several studies have shown that asthmatic patients with at least one variant allele (C/C or C/A) had a significantly better response to zafirlukast, montelukast, and pranlukast compared to the patients with the 2 wild type allele, as evidenced by increased FEV1, FVC and PEFR.

Leukotriene modifiers are the only class of commonly prescribed asthma medications that are administered orally and are subject to first pass effects. Although few studies have focused on genetic variants involved in leukotriene modifiers-metabolizing enzymes, it has been shown that there are significant interindividual differences in plasma levels of montelukast. Variants in SLCO2B1, a gene that encodes for the organic anion transporter OATP2B1, may partly mediate this variability. In fact, a nonsynonymous SNP in SLCO2B1 has been associated with plasma concentrations of montelukast after 1 and 6 months of treatment. Heterozygotes for the mutant allele demonstrated up to 30% reduction in their plasma montelukast levels compared to homozygotes for the wild-type alleles.
The existing pharmacogenetic studies on leukotriene modifiers are limited by their small sample sizes and lack of replication of their findings. Current data suggest that variants in ALOX5 and LTC4S likely modulate a portion of the variability in responses to these agents. Additional recently proposed candidates in the leukotriene pathway include TBXA2, PTGDR, and IL-13. However, replications of these findings in larger cohorts of asthmatics are warranted.

Future Directions

Many current studies are limited by their small sample size, imprecise phenotypic definitions, population stratification issues, multiple comparisons, and the lack of replication of the results. In addition, each genetic variant accounts for a minimal portion of the interindividual variability. Future studies should focus on these limitations. With the decreasing cost of high-throughput technologies, continued collaborations between genomic investigators are needed to perform and replicate large-scale genotyping and microarray studies, which will allow the identification of relevant and true association. Furthermore, future studies should incorporate multiple genetic variants and haplotype analyses to formulate a better prognostic model for response to asthma therapy.

Emergent areas in asthma pharmacogenomics include epigenetics and copy number variation (CNV). Epigenetics is the study of changes in gene expression patterns that are caused by mechanism other than changes in the nucleotide sequence of the genetic code itself and include DNA methylation, transcriptional regulation by small interfering RNAs (siRNAs) or micro RNAs (miRNAs), and post-transcriptional histone modifications. Although the field of asthma epigenetics is still in its early stages, a study of human bronchial biopsies showed that histone deacetylases (HDAC) activity was decreased in untreated asthmatics. Asthmatics receiving ICS had a higher level, but still lower than control subjects. These results support a role for epigenetics in asthma therapy, in particular with HDAC as a potential pharmacogenetic target.

CNVs are gains or losses of chromosome regions greater than 1 kb which can alter gene dosage. They can be inherited from parents or arise de novo and they have been implicated in the pathogenesis of childhood asthma. Specifically, in a genome-wide analysis of CNVs, children with allergic asthma were found to have decreased copy number at the TCRγ gene, which encodes for T-cell receptor gamma glycoprotein, a T-cell surface protein involved in cell-mediated immunity. Future studies are needed to replicate these findings.

Advances in statistical modeling will also aid in the creation of better predictive models. Asthma treatment response is a complex trait that is the result of many variables and genetic variants. Thus, integration of large-volume genetic data is needed in order to predict therapeutic response. The use of machine learning techniques such as Bayesian networks for data analysis may be useful in constructing future prediction models.

Conclusion

The field of pharmacogenetics has come a long way since Garrod developed the concept of “chemical individuality” at the beginning of the 20th century and so has the field of asthma pharmacogenetics. High-throughput technologies have also allowed the progression of candidate gene studies to large-scale genetic investigations. The integration of emergent fields of asthma pharmacogenomics such as epigenetics and copy number variations in future studies for a more comprehensive pharmacogenomics approach may help in the formulation of improved predictive models. This forms the basis for personalized asthma treatment, which will lead to improved health outcomes and more cost-effective care.
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Figure 1.
Distribution of the bronchodilator response across the three major asthma networks in SHARP. Note that, despite highly varying enrollment criteria, the overall distribution of response is remarkably similar between the clinical trial networks. This supports the ability to harmonize asthma pharmacogenetic phenotypes and the likely presence of common genetic determinants of response. ACRN=Asthma Clinical Research Network; CAMP=Children Asthma Management Program; CARE=Childhood Asthma Research and Education.
Figure 2.
Polymorphisms in ADRB2. Arg16Gly and Gln27Glu are located positions +47 and +79 respectively and are shown in the transmembrane β2-adrenergic receptor.
Figure 3.
Overview of the glucocorticoid pathway. The binding of glucocorticoid (GC) to the intracellular glucocorticoid receptor (GR) results in activation of the complex and dissociation of inhibitory proteins that usually keeps GR inactive. These include Hsp90, Hsp70 (heat shock proteins), the p23 phosphoprotein and FKBP52. The GC-GR complex translocates into the nucleus and alters transcription of genes involved in inflammation.
Figure 4.
Overview of the leukotriene pathway, demonstrating the site of action of anti-leukotriene therapies.