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Pharmacogenomics in Asthma Therapy: Where Are We and Where Do We Go?

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Abstract

The response to drug treatment in asthma is a complex trait and is markedly variable even in patients with apparently similar clinical features. Pharmacogenomics, which is the study of variations of human genome characteristics as related to drug response, can play a role in asthma therapy. Both a traditional candidate-gene approach to conducting genetic association studies and genome-wide association studies have provided an increasing list of genes and variants associated with the three major classes of asthma medications: β_2 -agonists, inhaled corticosteroids, and leukotriene modifiers. Moreover, a recent integrative, systems-level approach has offered a promising opportunity to identify important pharmacogenomics loci in asthma treatment. However, we are still a long way away from making this discipline directly relevant to patients. The combination of network modeling, functional validation, and integrative omics technologies will likely be needed to move asthma pharmacogenomics closer to clinical relevance.

INTRODUCTION

Asthma is characterized by chronic inflammation and smooth muscle dysfunction of the airway, which are associated with airway hyperresponsiveness (AHR) (1, 2). AHR leads to typical symptoms of asthma, such as recurrent episodes of wheezing, breathlessness, chest tightness, and coughing (see also The Asthma Phenotype, below). The global burden posed by asthma is considerable. More than 300 million people in the world currently suffer from asthma, and that number is estimated to become 400 million by 2025 (3). The number of disability-adjusted life years lost due to asthma worldwide is approximately 1% of the total and is similar to that for diabetes and liver cirrhosis (4). Moreover, the economic costs associated with asthma are estimated to exceed those of tuberculosis and HIV/AIDS combined (5).

The three major classes of asthma medications are β_2 -agonists, inhaled corticosteroids (ICS), and leukotriene modifiers. These primary therapies for asthma, used by most patients with the disease, focus on increasing bronchial smooth muscle relaxation (bronchodilation) and decreasing airway inflammation. The first class, the inhaled β_2 -agonists (both short and long acting), function as agonists of β_2 -adrenergic receptors (β_2 ARs) and are the most potent bronchodilators to date. β_2 -Agonists bind to the β_2 ARs, which are coupled via a stimulatory G protein to adenylyl cyclase, resulting in airway smooth muscle relaxation (6). Inhaled anticholinergics, which antagonize muscarinic cholinergic receptors, are also bronchodilators; however, they are less efficacious than inhaled β_2 -agonists. Recently, tiotropium bromide, a long-acting inhaled anticholinergic, was reported to have some additive bronchodilator effects on β_2 -agonists, especially in patients with severe asthma (7). Glucocorticoids (GCs) are the most potent anti-inflammatory agents for asthma, and they are administered as ICS, which constitute the second class of asthma medications. GCs cross the airway epithelial cell membrane, bind to glucocorticoid receptors (GRs) in the cytoplasm, and translocate to the nucleus. GRs homodimerize and bind to the GC-response element in GC-responsive genes; this GC-response element *trans*-activates genes encoding anti-inflammatory proteins (6). The third category of asthma medications is the leukotriene modifiers, which block the actions of leukotrienes such as the cysteinyl leukotrienes (CysLTs). CysLTs, which are synthesized from arachidonic acid by the enzyme 5-lipoxygenase (5-LO), cause bronchoconstriction, plasma exudation, and mucus secretion (8). Leukotriene modifiers block these leukotriene biochemical pathways by either inhibiting 5-LO or antagonizing the CysLT receptor 1 (CysLTR₁). **Figure 1** depicts the biochemical pathways involved in asthma treatment and the active mechanisms of these three types of asthma medications.

THE ASTHMA PHENOTYPE

Asthma is a disease characterized by reversible airflow obstruction that occurs either spontaneously or as a result of environmental stimuli, including drugs. The disorder is characterized by airway inflammation and epithelial damage and, in more severe cases, subepithelial fibrosis and smooth muscle hypertrophy. These pathologic changes, as mentioned above, result in reduced lung function, AHR, and the respiratory symptoms of wheezing, shortness of breath, and coughing. The asthma phenotype is heterogeneous and, as such, has many component parts. In terms of drug treatment, a wide range of phenotypes have been examined: exacerbations measured in multiple ways, symptoms, AHR, reduced lung function, and bronchodilator response (BDR). Each of these subphenotypes has a complex underlying pathobiology. At present, the number of clinically useful asthmatic subsets is unknown, and the best way to identify patients who would respond to specific treatments is still unclear. Because disordered steroid response and BDR are intrinsic to the disease, response to these drugs may be telling us something fundamental about

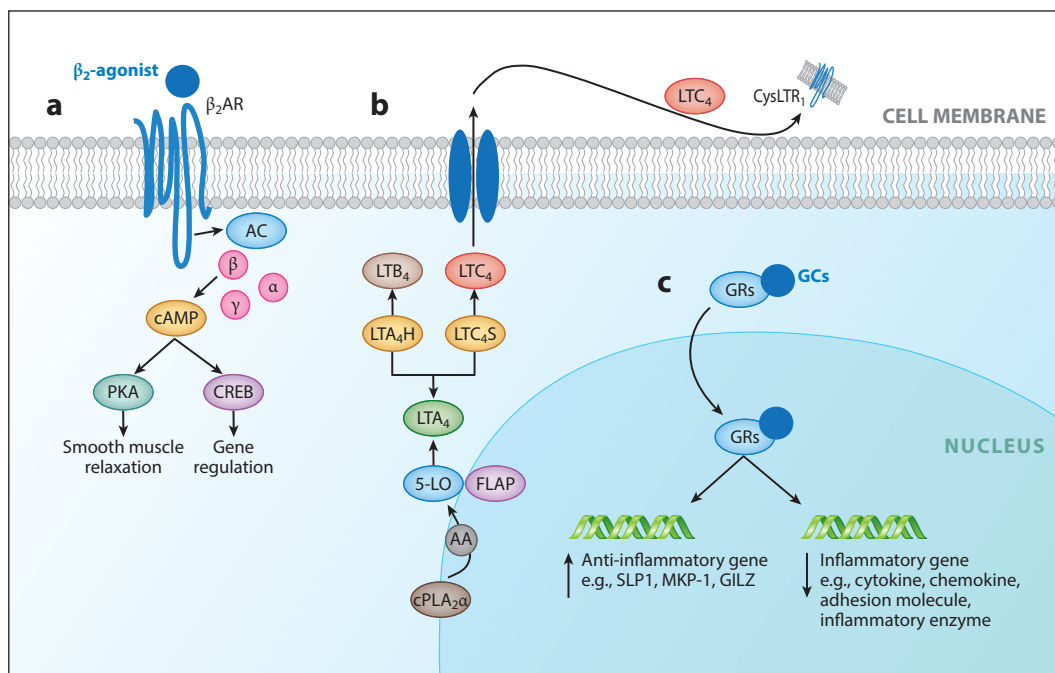


Figure 1

Pathways involved in asthma and the active mechanisms of asthma medications. (a) β_2 -Agonists bind to the β_2 ARs that are coupled via a stimulatory G protein to AC, resulting in airway smooth muscle relaxation via PKA and regulation of various genes via CREB. (b) Cell activation increases intracellular Ca^{2+} ions, leading to the association of $\text{cPLA}_2\alpha$ with the nuclear membrane and the generation of AA. This process activates the 5-LO that is located in the nuclear membrane and bound to FLAP. As a result, LTA_4 either is generated and converted to LTB_4 by LTA_4H or is conjugated with reduced glutathione by LTC_4S to form LTC_4 . LTC_4 is transported to the extracellular space, after which cysteinyl leukotriene receptor 1 (CysLTR₁) on target cells is activated. (c) GCs cross the epithelial cell membrane and bind to GRs in the cytoplasm, which translocate to the nucleus. GR homodimers bind to the GC-response element, which may *trans*-activate genes encoding anti-inflammatory proteins, such as SLPI, MKP-1, and GILZ. GRs also recruit histone deacetylase 2, which deacetylates core histones to suppress inflammatory gene transcription. Abbreviations: AA, arachidonic acid; AC, adenylyl cyclase; β_2 AR, β_2 -adrenergic receptor; cAMP, cyclic adenosine monophosphate; CREB, cAMP response element-binding; CysLTR₁, cysteinyl leukotriene receptor 1; cPLA_2 , cytosolic phospholipase A₂; 5-LO, 5-lipoxygenase; FLAP, 5-lipoxygenase activating protein; GC, glucocorticoid; GR, GC receptor; GILZ, glucocorticoid-induced leucine zipper; LTA_4 , leukotriene A₄; LTA_4H , leukotriene A₄ hydrolase; LTB_4 , leukotriene B₄; LTC_4 , leukotriene C₄; LTC_4S , leukotriene C₄ synthase; MKP-1, MAPK phosphatase 1; PKA, cyclic AMP-dependent protein kinase; SLPI, secretory leukoprotease inhibitor.

asthma and its various subcomponents. Whether there are ten asthmas, or only two or three, is currently unknown, but underlying pathways of drug response related to clinical subphenotypes may allow us to classify asthmatic subjects into subsets that are responsive to specific therapies. This is one of the main goals of asthma pharmacogenomics. Careful clinical phenotyping and the collection of multiple omics data types from relatively large numbers of patients will be necessary for meaningful stratification to occur.

WHY IS PHARMACOGENOMICS IMPORTANT IN ASTHMA?

The response to drug treatment in asthma is a complex trait and is markedly variable even in patients with apparently similar clinical features (9). Many studies have demonstrated interindividual variation in patient response to inhaled β_2 -agonist, ICS, and oral leukotriene modifier drugs

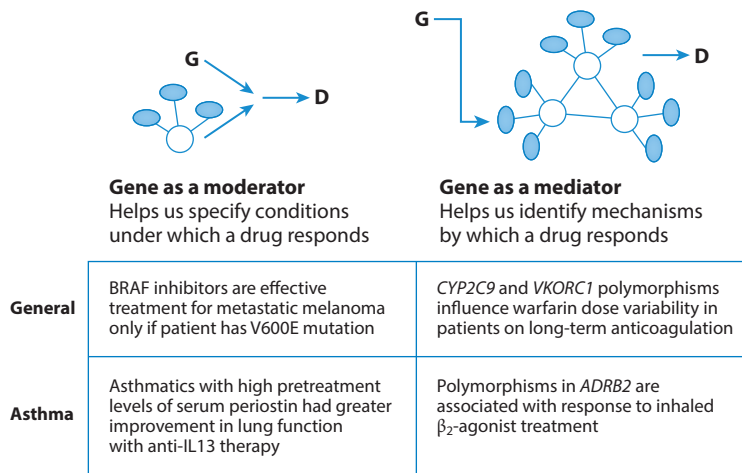


Figure 2

General purpose of a pharmacogenomics study. The goal of a pharmacogenomics study is to search the genome for genes that moderate or mediate drug responses; examples of general effects and effects on asthma are shown. A gene that functions as a moderator helps us to identify patients who might gain benefit from a drug or to specify conditions under which a drug works most effectively. A gene that functions as a mediator is involved in the pathophysiologic mechanisms by which a drug works and helps us to predict how a subject might respond to that drug. Abbreviations: D, drug response; G, gene.

(9–12). To measure heritability of drug response in patients with asthma, we used mixed-effects regression models to determine the intraclass correlation coefficient (ICC) for FEV1 (forced expiratory volume in 1 second; an indicator of airway obstruction) and methacholine PC₂₀ (dose of the inhaled methacholine that provokes a 20% drop in FEV1 compared with the value measured at baseline; an indicator of AHR) during repeated visits over a period of 4 years in the Childhood Asthma Management Program (CAMP) (13). The ICC for drug response compares the variability of the responses within and between individuals over the course of repeated drug administration and represents the proportion of the drug response that is innate and, therefore, most likely heritable. The ICC for FEV1 was 0.71 and that for PC₂₀ was 0.67 in the group treated with ICS. This high repeatability suggested that response to asthma medication has a genetic background. Other data from studies of families and twins support a role for heritability for both inhaled short-acting β_2 -agonists (SABAs; see below) and leukotriene modifiers (14).

The US Food and Drug Administration defined pharmacogenomics as “the study of variations in DNA and RNA characteristics as related to drug response” (15, p. 3). Pharmacogenetics focuses only on the DNA sequences of individual genes and is thought to be a subset of pharmacogenomics, which focuses on the whole genome and its components and regulators. Generally speaking, the purpose of a pharmacogenomics study is to search the entire genome for genes that moderate or mediate drug responses (**Figure 2**). A gene that functions as a moderator helps us to identify patients who gain benefit from a drug or to specify conditions under which a drug works most effectively. For example, the inhibitors of the oncogene *BRAF* were remarkably effective in patients with metastatic melanoma who harbored the V600E mutation in *BRAF* (16), and carbamazepine should not be administered to patients with Asian ancestry who carry the HLA-B*1502 allele if severe cutaneous adverse reactions are to be avoided (17). Periostin secreted from airway epithelial cells was first identified from a genomic study looking at asthma pathogenesis (18). However, a recent report showed that sputum periostin level is a surrogate marker for high Th2 status, the driver

for airway inflammation in asthma, and thus is useful in selecting asthmatic patients who respond to anti-interleukin-13 antibody treatment (19). By contrast, a gene that functions as a mediator is involved in the pathophysiologic mechanisms by which a drug works, and it helps us to predict how a subject might respond to that drug. For example, warfarin, a highly effective and commonly used anticoagulant, is metabolized primarily via oxidation in the liver by the gene *CYP2C9* and exerts its effect by inhibiting the protein vitamin K epoxide reductase complex subunit 1, which is encoded by the gene *VKORC1* (20). As expected, genetic polymorphisms in both genes play key roles in mediating the effect of warfarin therapy on coagulation (21) and have led to genotype-guided dosing tables in warfarin labeling (22). The β_2 AR pathway is another example of mediation in that polymorphisms in the *ADRB2* gene mediate response to β_2 -agonist drugs. So far, nearly 49 validated genetic polymorphisms in the *ADRB2* gene, which encodes the ADRB2 receptor, have been identified in asthmatic subjects (23). Among them, a common variant coding for either a glycine or an arginine in the sixteenth amino acid position (Arg16Gly) was reported to be associated with differences in the extent of receptor downregulation in response to β_2 -agonist treatment in vitro (24). Subsequently, the BARGE (Beta-Adrenergic Response by Genotype) trial—the first genotype-stratified, prospective placebo-controlled trial of asthma treatment—found that subjects with Arg/Arg had lower peak expiratory flow rates (PEFRs) during treatment with regular albuterol compared with placebo (25). Although the Arg/Gly polymorphism is functional in vitro, whether the polymorphism is primarily responsible for the in vivo effects observed with the medication is unclear. More functional studies on the variants in this gene are needed. (For details, see Candidate-Gene Approach, below.)

WHERE ARE WE?

All genes harbor variants such as single-nucleotide polymorphisms (SNPs) and copy-number variations. However, selecting the genetic variants that are most relevant in terms of their influence on drug treatment response is challenging. So far, three approaches have been used to address this challenge. The first is a candidate-gene approach driven by an a priori hypothesis based on the pharmacokinetic and/or pharmacodynamic properties of a drug. The second approach is the hypothesis-free genome-wide association study (GWAS), which became possible owing to progress in genotyping technology. The third approach, which has led to a more holistic view of pharmacogenomics, is an integrative systems biology approach that combines large-scale molecular profiling traits (e.g., transcriptomic, proteomic, metabolomic traits) and genetic variants. In this section, we provide some examples of genetic variants associated with response to asthma medications that were revealed by these three methodologic approaches (see **Table 1**).

Candidate-Gene Approach

Inhaled β_2 -agonists are widely used in asthma treatment for the purpose of bronchodilation and are divided into two classes according to their onset of action and duration of action. Short-acting β_2 -agonists (SABAs) are recommended for relief of acute symptoms caused by bronchospasm, whereas long-acting β_2 -agonists (LABAs) are used in conjunction with ICS to maintain long-term control of asthma (26). Inhaled β_2 -agonists act by binding to β_2 ARs. The sequence of the β_2 AR has been known for many years, and the effect of genetic variants in the *ADRB2* gene on receptor function has been intensively examined (24, 27, 28). For this reason, pharmacogenetic studies examining the heterogeneity in patient response to inhaled β_2 -agonists have been dominantly focused on *ADRB2*. For the Arg16Gly variant in the *ADRB2* gene, Gly16 caused a greater reduction (downregulation) of ADRB2 than did Arg16 in cultured primary human smooth muscle

cells after incubation with isoproterenol (a SABA) (24). However, Israel et al. (29) reported that regular use of inhaled β_2 -agonists was associated with a decline in morning and evening PEFs in subjects who were homozygous for Arg16 but not in subjects with other genotypes. To explain this seemingly contradictory finding, the authors postulated that the subject with Gly/Gly would

Table 1 Approaches utilized in asthma pharmacogenomics: studies and examples

Drug class	Gene	Associated variant(s)	Comments (Reference)
Candidate-gene approach			
Inhaled β_2 -agonists	<i>ADRB2</i>	rs1042713 (Arg16Gly)	Drug receptors have been thought to be optimal targets in pharmacogenomics studies (25).
	<i>ARG1</i>	rs2781659	Given that a response to inhaled β_2 -agonists in asthma is a complex phenotype, the authors selected targets by screening variants in the β_2 -adrenergic pathways (32).
	<i>THRB</i>	rs892940	This study hypothesized that genes regulated by in vitro exposure to isoproterenol (β_2 -agonist) would be good candidates to predict a response to inhaled β_2 -agonists; thus, the authors tested transcription factor genes (34).
Inhaled corti- costeroids	<i>CRHR1</i>	rs242941, rs1876828, rs2236647, rs6591838	The authors focused on the endogenous glucocorticoid biosynthesis pathway in selecting optimal targets (35).
	<i>TBX1</i>	rs2240017 (His33Glu)	This study showed that drug responses could be related to genes that are not in the direct metabolic or signaling pathway of the drug (43).
Leukotriene modifiers	<i>ALOX5</i>	Promoter repeat rs4987105, rs4986832	The authors focused on the leukotriene biosynthesis pathway in selecting optimal targets (45, 46).
	<i>CYSLTR2</i>	rs91227, rs912278	
GWAS approach			
Inhaled β_2 -agonists	<i>SPATS2L</i>	rs295137	This study consisted of 1,644 asthmatic subjects from six trial cohorts and two replication cohorts (48).
	<i>SLC22A15</i>	rs1281748, rs1281743	A total of 2,313 Latino children with asthma were enrolled in the primary and replication studies. The results suggested that a rare variant contributes to a differential drug response (50).
Inhaled corti- costeroids	<i>GLCCII</i>	rs37972	118 probands from the CAMP trial and 935 asthmatic subjects from four additional replication cohorts confirmed this association (51).
	<i>T</i>	rs3127412, rs6456042	This study consisted of 408 asthmatic patients from the CAMP, ACRN, and CARE trials and a replication cohort consisting of 407 asthmatic patients (55).
	<i>FBXL7</i>	rs10044254	This is the first GWAS to examine response of asthma symptoms to ICS. The result suggested that there may be a specific genetic mechanism regulating symptomatic response in children that does not occur in adults (56).
Leukotriene modifiers	<i>ALOX5AP</i>	rs17238738	This is the first GWAS examining a leukotriene modifier in asthma. It showed that previously unreported genes and loci within pathways for leukotriene production and response are implicated in therapeutic responsiveness (A. Dahlin, A. Litonjua, C.G. Irvin, S.P. Peters & J.J. Lima et al., submitted manuscript).
	<i>RGS13</i>	rs4147217	

(Continued)

Table 1 (Continued)

Drug class	Gene	Associated variant(s)	Comments (Reference)
Integrative systems biology approach			
Inhaled β_2 -agonists	15 genes	15 SNPs	The authors aimed to validate a genetic predictive model of BDR created with Bayesian networks and constructed a model using 15 SNPs in 15 genes with the highest likelihood of predicting BDR (63).
Inhaled corticosteroids	<i>TNC</i>	rs7850103	The authors reported that drug-specific eQTLs associated with changes in <i>TNC</i> expression following dexamethasone stimulation in cultured human osteoblast-like cells were also associated with clinical response to ICS (65).
	<i>SPATA20</i>	rs6504666, rs1380657	This is an eQTL analysis in dexamethasone-treated and dexamethasone-untreated LCLs obtained from asthmatic subjects in the CAMP trial. The authors tested the association of eQTLs with longitudinal change in airway responsiveness to methacholine in subjects on ICS (66a).
	<i>ACOT4</i>	rs12891009	
	<i>BRWD1</i>	rs2037925, rs2836987	
	<i>ALG8</i>	rs1144764	
	<i>NAPRT1</i>	rs3793371	

Abbreviations: ACRN, Asthma Clinical Research Network; BDR, bronchodilator response; CAMP, Childhood Asthma Management Program; CARE, Candidate Gene Association Resource; GWAS, genome-wide association study; ICS, inhaled corticosteroid; SNP, single-nucleotide polymorphism.

already have reduced expressions of *ADRB2* at baseline; thus, the subjects with Arg/Arg would be more susceptible to the desensitizing effect of regular β_2 -agonist use. As mentioned above, the subjects with the Arg/Arg genotype showed lower PEFs compared with placebo when they were treated with regular SABA (albuterol) in the prospective, genotype-stratified study (25), although the difference was small [10 L/min (range: 2–19 L/min)] and of a magnitude of questionable clinical significance. The effect of the Arg16Gly locus on response to LABA was evaluated in two large, genotype-stratified randomized controlled trials. Open-label use of ICS was allowed in the study performed by the National Heart, Lung, and Blood Institute Asthma Clinical Research Network (30), as LABA is used in combination with ICS in the usual care of asthmatic patients. In this study, both Arg/Arg and Gly/Gly homozygous subjects showed improved PEFs after treatment with LABA (salmeterol), although there was no bronchoprotection in Arg/Arg subjects. The subsequent and larger study included the LABA (salmeterol)-only arm in addition to the ICS/LABA combination arm to test the possibility that corticosteroids mask pharmacogenetic interaction of *ADRB2* variants with LABA (31). There was no effect of the Arg16Gly locus on the therapeutic efficacy of the ICS/LABA combination and also no evidence of a masking effect by ICS.

The data on the Arg16Gly locus in the *ADRB2* gene raised two important points to be considered. First, the clinical meaning of the effect of genetic variants generated from in vitro experiments should be tested via a confirmatory human trial with prospective design whenever possible. For example, the reported frequency of the minor allele of β_2 ARs (Gly16) for Arg16Gly in Caucasians is approximately 40% (23). Second, the pharmacogenetic effect of any individual variant is likely to be small. Of note, other functional regulatory variants in the *ADRB2* gene have not been evaluated in human studies.

In any human pharmacogenetic study, practical strategies to select candidate genes are to focus on pathways or to utilize information generated from other genomic studies. To screen variants in candidate genes in the β_2 AR pathway, we genotyped 844 SNPs in 111 candidate

genes in 209 children and their parents who were participants in the CAMP trial. We identified a novel gene, arginase 1 (*ARG1*), that is associated with BDR to inhaled SABA in children and adults with asthma (32). A previous study showed that isoproterenol evoked changes ($\geq 50\%$ difference) in transcription factor gene expression in human airway epithelial and smooth muscle cell lines under a proasthmatic culture milieu (33). We hypothesized that genes regulated by in vitro exposure to isoproterenol would be good candidates for determining response to β_2 -agonists and tested 1,116 SNPs in 98 of those transcription factor genes for association with BDR in asthmatic patients (34). We found that a SNP 5' from the thyroid hormone receptor beta (*THRB*) gene was associated with BDR in the childhood population and in two adult populations.

Using an approach similar to what we employed to evaluate inhaled β_2 -agonists, we focused on the GC biosynthesis pathway and screened 131 SNPs in 14 genes. We then searched for the association between our SNPs and the response to an 8-week treatment with ICS, as measured by percent change in FEV1 in three independent asthmatic populations. We identified the SNP rs242941, in the corticotropin-releasing hormone receptor 1 (*CRHR1*) gene, that showed consistent, significant associations in multiple populations (35). Corticotropin-releasing hormone binds to *CRHR1* in the pituitary gland and modulates adrenocorticotrophic hormone-induced cortisol production. Therefore, asthmatics with inhibitory alterations in this gene would be expected to have reduced capacity to secrete cortisol and consequently would be more responsive to GCs administered exogenously. However, a subsequent study reported that three haplotype-tagging SNPs in *CRHR1* were not associated with the long-term FEV1 decrease in adults using GCs (36). Two features may explain this discrepancy in the results regarding the association. First, the target phenotypes of the two studies (FEV1 increase in response to 8-week treatment with ICS versus FEV1 decrease in response to treatment with ICS for more than 10 years) were different. The complexity of response to asthma medication suggests that there may be multiple pharmaceutical pathways that involve different genes. For example, we found that genetic predictors of a poor long-term response to ICS differed markedly depending on the definition of outcome (exacerbation versus improved lung function) (37). Second, there might be other genetic factors in addition to *CRHR1* that determine the response of lung function to ICS. For example, the chromosome 17q21 locus harboring the *ORMDL3*, *GSDML*, *ZBPB2*, and *IKZF3* genes has been reported to be related to asthma pathogenesis or ICS response (38–40). We demonstrated that the *CRHR1* SNP was in strong linkage disequilibrium with a 900-kb inversion on chromosome 17q21 (41). Therefore, the *CRHR1* response to ICS might result from long-range linkage disequilibrium with another gene in the chromosome 17q21 region. GR and nuclear transcriptional factors related to T cell differentiation have also been found to be candidates for ICS treatment response. Hawkins et al. (42) evaluated the effect of SNPs in eight GC-complex genes on ICS response and found that rs6591838 in the *STIP1* gene was significantly associated with percent change in FEV1 after 4 weeks of ICS treatment. Tantisira et al. (43) reported that rs2240017, a nonsynonymous coding SNP in the *TBX21* gene, was associated with marked improvement in AHR in asthmatic children treated with ICS for 4 years. *TBX21* codes for the nuclear transcription factor T-bet, which is responsible for differentiating Th1 cells from naive T cells while simultaneously repressing Th2 cells (44).

As mentioned above, leukotriene modifiers block leukotriene pathways by inhibiting 5-LO or by antagonizing CysLTR₁ (**Figure 1**). Therefore, the *ALOX5* gene coding for the 5-LO and the *CYSLTR1* and *-2* genes coding for CysLTR₁ and CysLTR₂ have long been the candidate genes for responses to treatment with leukotriene modifier drugs. A tandem repeat polymorphism (factor Sp1-binding motif) within the promoter region of the *ALOX5* gene was reported to be associated with an improvement of 19–23% in FEV1 in a clinical trial of the 5-LO inhibitor

ABT-761 (45). Klotsman et al. (46) showed that two SNPs in the *CYSLTR2* gene (rs91227 and rs912278) and two SNPs in the *ALOX5* gene (rs4987105 and rs4986832) were associated with an 18–25% improvement in PEFR in response to 12-week treatment with montelukast. Interestingly, pharmacogenetic loci associated with the response to CysLT receptor antagonists also influenced the response to zileuton, a 5-LO inhibitor (47). This result suggested that investigators might need only a single genetic marker to predict the response in a particular therapeutic pathway rather than needing individual markers for each class of medication belonging to that pathway.

Genome-Wide Association Study Approach

A GWAS is a study that is designed to find genetic variations associated with observable phenotypes by scanning SNP markers that tag via linkage disequilibrium the complete genomes of many people, usually diseased or affected subjects and a set of controls. Thus, for example, a GWAS of BDR has the potential to provide new insight into its pharmacogenomics. Himes et al. (48) performed a GWAS in 1,644 non-Hispanic Caucasian asthmatics and found that the variant with the statistically greatest association was located near the spermatogenesis-associated serine-rich 2-like (*SPATS2L*) gene. In this study, the authors replicated this finding in 1,051 Caucasian subjects from two independent cohorts and also provided functional data that suggested *SPATS2L* may regulate BDR by showing that the knockdown of *SPATS2L* mRNA in human airway smooth muscle cells increased β_2 AR levels. Another GWAS that analyzed BDR at entry and longitudinally in a long-term randomized clinical trial identified an intergenic SNP, rs11252394, that is located near multiple genes previously correlated with lung inflammation (49). However, the biological significance of this variant in modulating BDR to inhaled β_2 -agonists needs to be elucidated. A recent GWAS of BDR performed in 1,782 Latino children with asthma shed light on the role of rare variants in asthma pharmacogenomics (50). The authors observed more small p values than expected by chance in the quantile-quantile plot. However, this excess of small p values disappeared after the removal of SNPs that had minor allele frequencies of less than 5%, suggesting that the excess was driven by the inclusion of rare variants. The rare variants identified were located in the proximity of solute carrier (*SLC*) genes, including two rare variants in the *SLC22A15* gene, which is expressed in the lung and bronchial epithelial cells. A GWAS of BDR to inhaled β_2 -agonists has identified novel genetic predictors other than variants in the *ADRB2* gene, which previously has been the only target for the study of BDR pharmacogenetics. Given that BDR to inhaled β_2 -agonists is influenced by multiple genes, investigating the effects and potential interaction of multiple genes is necessary. Each of these genes may have a small but significant effect; thus, synergism may exist between different genetic variants. As GWASs can help in the investigation of common variants, they will remain an important component of pharmacogenomics so that investigators can uncover the small to moderate effects of individual genetic determinants of drug response.

GWASs also have identified novel pharmacogenetic loci associated with the response to ICS. A SNP in the promoter of the glucocorticoid-induced transcript 1 gene (*GLCC1*), rs37972, was associated with lung functional response to ICS treatment in four independent populations (pooled p value = 0.0007) (51). Lymphoblastoid B cells (LCLs), created by transforming B lymphocytes with Epstein-Barr virus, have been used for functional analysis of genetic variations (52–54). Interestingly, the dexamethasone-induced expression of the *GLCC1* gene was significantly lower in the LCLs from subjects who were homozygous for the mutant allele of rs37972 compared with those who were homozygous for the wild-type allele. This study (51) showed the possibility that cell lines derived from subjects enrolled in clinical trials can serve as an in vitro assay platform for predicting drug response. Another GWAS of 418 asthmatic subjects from three independent

clinical trials identified two SNPs in the *T* gene (rs3127412 and rs6456042) associated with pulmonary function response to ICS, a finding replicated in additional asthmatic subjects treated with ICS (55).

GWAS results may reveal novel mechanisms underlying drug response. For example, we have identified a novel SNP, rs10044254, that is associated with decreased expression of the *FBXL7* gene and improved symptomatic response to ICS (56). Our results were replicated in another pediatric asthmatic cohort, but not in adult asthmatic cohorts, suggesting that this may be a specific genetic mechanism regulating symptomatic response to ICS in children but not adults.

The first GWAS to examine response to leukotriene modifiers was conducted recently (A. Dahlin, A. Litonjua, C.G. Irvin, S.P. Peters & J.J. Lima et al., submitted manuscript). Dahlin et al. performed a gene-environment interaction GWAS of the 3-month change in FEV1 following zileuton (5-LO inhibitor) treatment [the treatment group (zileuton or placebo) was specified as the interaction term]. The authors then tested the replicability of their results in a second GWAS of the main effects of zileuton, as well as in two independent GWASs examining the response to montelukast (CycLT₁ blocker). Two SNPs (rs4147217 in the gene *RGS13* and intergenic rs347967) replicated in all four cohorts. This gene and these SNPs have not been reported previously, although they are within pathways for leukotriene production and response and suggest the importance of GWASs in discovering new pharmacogenetic loci.

Integrative Systems Biology Approach

Recent advances in omics technology now provide a large amount of data. The complementary nature of the data makes them more informative, particularly when they are integrated (57). In pharmacogenomics research, investigators can benefit from the application of a systems biology approach, which involves the iterative and integrative study of biological processes and findings, including response to perturbations (58, 59). An integrative systems biology approach can be summarized as follows: (a) Global genomic, transcriptomic, proteomic, and metabolomic data sets are integrated with biologic and clinical data; (b) predictive models are generated through the use of data mining and network reconstruction methods along with machine-learning algorithms; (c) predictive values of a generated model are validated through resampling or replication in external and independent data sets; and (d) a final model can then be used to predict behavior of the network of interest or to allow the network to accommodate new information by perturbation. Recently, several investigators have begun to use methodologies from integrative systems biology to predict response to asthma medications.

Examples of machine-learning algorithms that can be useful for descriptive and predictive modeling are Bayesian networks (60, 61). They consist of nodes (biological entities such as SNPs, genes, proteins, or phenotypes) and edges (connections or conditional dependencies between the nodes) and are presented by directed acyclic graphs of a joint probability distribution of the nodes (61, 62). For example, Himes et al. (63) tried to validate a genetic predictive model of BDR created with Bayesian networks. They used a set of 426 SNPs from 254 candidate genes based on previously published associations with asthma or asthma treatment derived from the genotyping of 308 subjects in the CAMP trial. The authors then constructed a model using 15 SNPs in 15 genes with the highest likelihood of predicting BDR. This model showed fairly good accuracy (5-fold cross-validation area under a receiver-operating characteristic curve of 0.75) that was superior to the accuracy yielded by a single-gene approach. The analysis revealed possible limitations of this model—it had reduced power to predict genetic effects (i.e., its small sample size was moderately outnumbered by the number of SNPs) and a lack of external validation—but this study showed a potential benefit of Bayesian networks in pharmacogenomics.

An expression quantitative trait locus (eQTL) is a genomic locus that regulates expression levels of mRNAs. It can be used to enrich or refine GWAS results and to develop models through various systems biology approaches (64). Grundberg et al. (65) reported that drug-specific eQTLs associated with changes in tenascin C (*TNC*) gene expression following dexamethasone stimulation in cultured human osteoblast-like cells were associated with clinical response to ICS in asthma. As mentioned above, LCLs have traditionally been used for acquiring gene expression data. If these cell lines come from the participants in a clinical trial, a direct link between the in vitro and in vivo experiments strengthens the validity of results derived from an integrative approach (66). We conducted an eQTL analysis using more than 300 subjects with gene expression data for dexamethasone-untreated and dexamethasone-treated LCLs obtained from asthmatic subjects in the CAMP trial, and we tested the associations of eQTLs with longitudinal change in airway responsiveness to methacholine (LnPC₂₀) in subjects on ICS (66a). We found a striking overrepresentation of LnPC₂₀-associated *cis*-eQTLs—190 SNPs—among differentially expressed genes in Caucasians in the CAMP cohort. Forty-six of these 190 clinical associations were replicated in African Americans in this cohort; these associations included seven SNPs near six genes that showed genome-wide significance. Notably, the majority of those findings would not have been detected by analysis of the samples that were untreated, suggesting that identifying eQTLs after relevant environmental perturbations makes it possible to reveal pharmacogenetic variants.

WHERE DO WE GO FROM HERE?

As reviewed above, there has been substantial progress in asthma pharmacogenomics. However, we are still far from making this discipline directly relevant to patients. There are several reasons for this translational gap. First, in pharmacogenetics studies to date, we have been identifying genes one at a time, and the genes we have found are predominantly regulatory in their functional effects and thus explain only a small part of the variability in the drug response phenotype. We often are not able to expand the sample size in pharmacogenetics research because most clinical trials are small and involve a fixed number of subjects, so obtaining large numbers of subjects for GWASs is difficult. Second, epigenetic changes can modify genetic effects in time-, environment-, and tissue-specific manners. Third, genes interact epistatically (i.e., together) in networks, and consideration of this gene-gene interaction is critical for expanding the proportion of phenotypic variance that can be attributed to genetics. Moreover, nongenetic components including gender, nutrients, lifestyle, and even intestinal microflora can significantly interact with genetics to determine drug response phenotype. The roles of all such factors are still unknown in asthma pharmacogenomics. Finally, functional and clinical validations of identified genetic variations are critical for creating personalized medicine. However, solutions to these challenging problems are already in sight. Below, we discuss our vision of the destination to which we are headed in order to reach a translational endpoint in asthma pharmacogenomics.

Propagation of Pharmacogenomics Effects Through Networks

In biological systems, genes, proteins, and metabolites interact with one another to result in a physiologic function at the cellular and organ levels. This higher-order, complex interaction among molecules can be visualized as a network. As with the Bayesian networks mentioned above, a network typically consists of nodes representing biological entities (e.g., gene, protein, disease) and edges representing the relationships among them (e.g., physical interaction, coexpression, shared pathway). Robustness is a key property of biological networks that enables the maintenance of

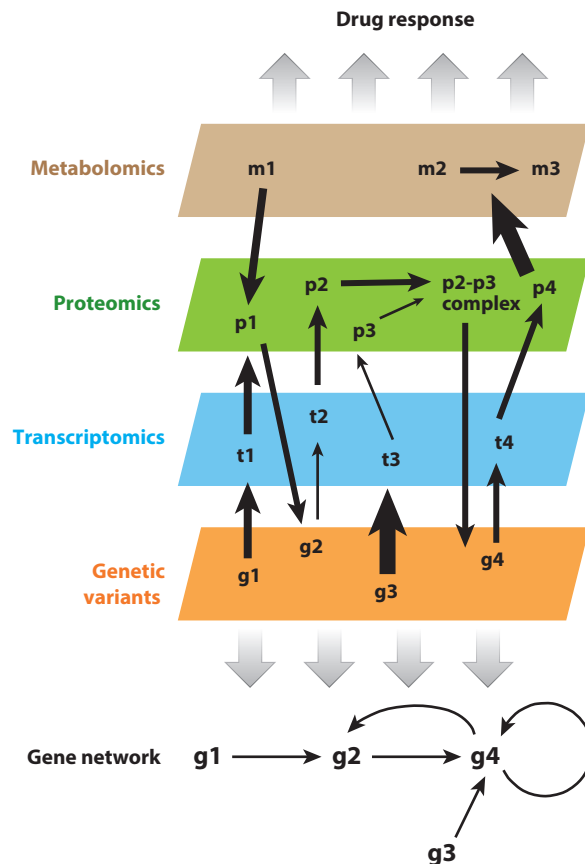


Figure 3

Gene network identified by an integrative systems biology approach. An integrative systems biology approach explores the interactions among genetic variants (g) and multiple types of omics data including transcripts (t), proteins (p), and metabolites (m) in a network context. Interactions are shown as arrows, and the thickness of an arrow represents the strength of interaction. On the basis of the interactions among those traits, a directional gene network of g1–4 can be inferred. Figure modified from Reference 82 with permission.

function in the face of external and internal perturbations (67, 68). We believe that an integrative systems biology approach is the best way to apply networks in asthma pharmacogenomics (Figure 3). In this approach, multiple types of omics data are integrated in a network context, and a data-driven network is constructed using computational algorithms, such as linear and nonlinear correlations, graphical Gaussian modeling, Bayesian networks, or their combinations (69). So far, most studies, including those mentioned in this review, have related genetics to a single omics platform. However, we believe that the comprehensive integration of genetics with multiple types of omics data will be the most desirable approach. An integrative personal omics profile proposed by Chen et al. (70) is a promising example. The investigators performed a longitudinal analysis of data derived from multiple-omics analysis in a single individual, including whole-genome DNA sequencing as well as genomic, transcriptomic, proteomic, metabolomic, and autoantibody profiling. This individual had two viral infections and developed type 2 diabetes mellitus over a 14-month period of observation. The authors found extensive, dynamic changes in diverse molecular

components and biological pathways during both the healthy and disease phases of the individual's life.

Gene-Environment Interaction

Epigenetic regulation may be involved (71) in asthma pathogenesis. There are three known mechanisms for epigenetic regulation: CpG (regions of DNA where a cytosine nucleotide occurs next to a guanine nucleotide in the linear sequence of bases) methylation, histone modification, and RNA-associated silencing. Methylation of the C⁵ family of cytosine residues in DNA has long been recognized as an important silencing mechanism of genes (72, 73) and is the most well understood epigenetic mechanism because of the early availability of high-throughput technology. Changes in promoter methylation status can initiate or slow gene expression, which, in turn, may affect response to drugs. microRNAs (miRNAs) are single-stranded, 21–23-nucleotide-long, independent functional units of noncoding RNA that can inhibit the translation of proteins and destabilize their target mRNAs by binding to the target transcript in the 3' untranslated region (74–76). Recent reports have showed that miRNAs can regulate pharmacology-related genes and drug responses in the treatment of cancer (77), cardiovascular disease (78), and diabetes (79). To date, though, we have not seen the publication of a pharmacogenomic study that evaluates the role of methylation or miRNA in predicting drug response in asthma. However, in other settings, genes related to dexamethasone resistance in multiple myeloma were identified through genomic screening that revealed genes silenced by DNA methylation (80). Such screening also implicated downregulation of miR-128b and miR-221 in GC resistance in acute lymphocytic leukemia (81). Therefore, epigenetic regulation likely contributes to drug treatment response in asthma.

The change in global gene expression in human LCLs in response to perturbations can be a convenient tool for studying the nature of gene-environment interactions (82). Vitamin D was recently reported to modify the response to ICS (83, 84). To explore the potential interaction between vitamin D and gene expression, one could evaluate dexamethasone-induced changes in gene expression in LCLs incubated in the absence or presence of vitamin D (in this case, vitamin D is an environmental perturbation).

Functional and Clinical Validation

The critical step to using genomic variants in clinical practice is validation, which can be evaluated from two perspectives: functional and clinical relevancy. The workflow for functionally analyzing and interpreting genomic variants obtained from GWASs consists of the fine mapping of variants, in silico annotation, the identification of a target gene by proving the functional relevancy of variants, and the evaluation of target gene function in the context of pathogenesis. This approach is nicely summarized in a recent review paper by Edwards et al. (85). Bioinformatics aids that can help determine the functional relevancy of variants are online resources and publicly available large-scale genome-wide data sets, such as the ENCyclopedia Of DNA Elements project (ENCODE; <http://www.genome.gov/10005107>), the Nuclear Receptor Cistrome database (http://cistrome.org/NR_Cistrome/), and the National Institutes of Health Roadmap Epigenomics Mapping Consortium (<http://www.roadmapepigenomics.org/>). For the experimental approaches, tissue-specific eQTL analysis, chromatin immunoprecipitation sequencing (ChIP-Seq), and DNaseI hypersensitive site sequencing can be utilized. To evaluate the function of target genes, inducing or silencing effects can be measured in cultured cell lines, human tissue, and animal models, including mice. A recent study by Zhou et al. (86) is a good example of the use of this approach. They performed gene expression analysis in a human bronchial epithelial cell line after silencing the Hedgehog interacting protein (*HHIP*) gene, one

of the genes implicated in the pathogenesis of chronic obstructive pulmonary disease (87). The authors found 296 genes with differential expression that were enriched for variants nominally associated with the disease. Network modeling demonstrated that the extracellular matrix and cell proliferation genes influenced by *HHIP* tended to be interconnected.

Functional validation of networks is still a subject of scientific debate. Network validation involves evaluating the concordance of predictions made from the network and corresponding experimental outcomes. For this, one can intentionally perturb the network (e.g., knock out a hub gene) and observe the results. The performance of algorithms in constructing networks from data may be quantified in the context of random sampling. Measuring the distance between two networks generated from primary and replication analyses is also useful for validation (88).

The clinical relevance of genomic variants also needs to be determined. A retrospective analysis can provide important supporting evidence if good design principles are applied (89). Biological samples collected in clinical trials enabled us to assess new pharmacogenomic hypotheses rapidly, as we learned from studies of samples from the CAMP trial (90). However, the question of whether prospective, genotype-stratified trials are necessary prior to implementation of pharmacogenetic testing is still controversial (91). A potentially effective approach might be the use of adaptive clinical trials, with pharmacogenomics included as part of the main trials (92).

CONCLUSION

Asthma pharmacogenomics is unique in that the medications used to treat the disease are part of the pathophysiologic process of the disease. In other words, genes that contribute to asthma itself are likely to be asthma pharmacogenomics genes and vice versa. Studies that leverage analyses of fetal lung development genomics and asthma genetics should enhance the field of asthma pharmacogenomics. Although much progress has been made, asthma pharmacogenomics is still not able to predict clinical outcomes with sufficient precision to be of clinical utility. The combination of network modeling, functional validation, and integrative omics technologies will likely be needed to move asthma pharmacogenomics closer to clinical reality.

Critics of the field of pharmacogenomics generally, and of asthma pharmacogenomics specifically, think that advances in defining responders and nonresponders have not progressed significantly since the sequencing of the human genome. They think that further work on network modeling, functional validation of these networks, and the regulatory variants that have been identified as a result of GWASs and network modeling will be futile in defining novel therapies and in determining who will respond to existing therapies. We respectfully disagree with this view. We think that the gene networks for asthma and asthma drug treatment significantly overlap and that by looking carefully at these pathways, we may not only find better and novel treatments but also find a cure for the disease.

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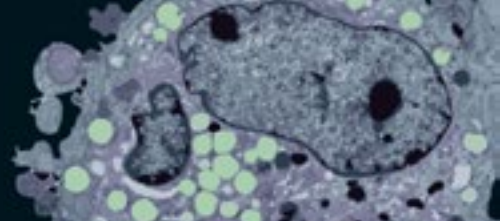
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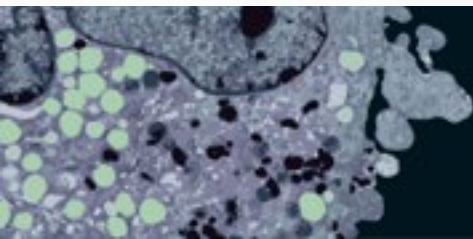
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TABLE OF CONTENTS FOR VOLUME 1:

- ***How Tumor Virology Evolved into Cancer Biology and Transformed Oncology***, Harold Varmus 
- ***The Role of Autophagy in Cancer***, Naiara Santana-Codina, Joseph D. Mancias, Alec C. Kimmelman
- ***Cell Cycle–Targeted Cancer Therapies***, Charles J. Sherr, Jiri Bartek
- ***Ubiquitin in Cell-Cycle Regulation and Dysregulation in Cancer***, Natalie A. Borg, Vishva M. Dixit
- ***The Two Faces of Reactive Oxygen Species in Cancer***, Colleen R. Reczek, Navdeep S. Chandel
- ***Analyzing Tumor Metabolism In Vivo***, Brandon Faubert, Ralph J. DeBerardinis
- ***Stress-Induced Mutagenesis: Implications in Cancer and Drug Resistance***, Devon M. Fitzgerald, P.J. Hastings, Susan M. Rosenberg
- ***Synthetic Lethality in Cancer Therapeutics***, Roderick L. Beijersbergen, Lodewyk F.A. Wessels, René Bernards
- ***Noncoding RNAs in Cancer Development***, Chao-Po Lin, Lin He
- ***p53: Multiple Facets of a Rubik's Cube***, Yun Zhang, Guillermina Lozano
- ***Resisting Resistance***, Ivana Bozic, Martin A. Nowak
- ***Deciphering Genetic Intratumor Heterogeneity and Its Impact on Cancer Evolution***, Rachel Rosenthal, Nicholas McGranahan, Javier Herrero, Charles Swanton
- ***Immune-Suppressing Cellular Elements of the Tumor Microenvironment***, Douglas T. Fearon
- ***Overcoming On-Target Resistance to Tyrosine Kinase Inhibitors in Lung Cancer***, Ibiayi Dagogo-Jack, Jeffrey A. Engelman, Alice T. Shaw
- ***Apoptosis and Cancer***, Anthony Letai
- ***Chemical Carcinogenesis Models of Cancer: Back to the Future***, Melissa Q. McCreery, Allan Balmain
- ***Extracellular Matrix Remodeling and Stiffening Modulate Tumor Phenotype and Treatment Response***, Jennifer L. Leight, Allison P. Drain, Valerie M. Weaver
- ***Aneuploidy in Cancer: Seq-ing Answers to Old Questions***, Kristin A. Knouse, Teresa Davoli, Stephen J. Elledge, Angelika Amon
- ***The Role of Chromatin-Associated Proteins in Cancer***, Kristian Helin, Saverio Minucci
- ***Targeted Differentiation Therapy with Mutant IDH Inhibitors: Early Experiences and Parallels with Other Differentiation Agents***, Eytan Stein, Katharine Yen
- ***Determinants of Organotropic Metastasis***, Heath A. Smith, Yibin Kang
- ***Multiple Roles for the MLL/COMPASS Family in the Epigenetic Regulation of Gene Expression and in Cancer***, Joshua J. Meeks, Ali Shilatifard
- ***Chimeric Antigen Receptors: A Paradigm Shift in Immunotherapy***, Michel Sadelain





Contents

A Conversation with Susan Band Horwitz <i>Susan Band Horwitz and I. David Goldman</i>	1
Introduction to the Theme “Precision Medicine and Prediction in Pharmacology” <i>Paul A. Insel, Susan G. Amara, and Terrence F. Blaschke</i>	11
Identifying Predictive Features in Drug Response Using Machine Learning: Opportunities and Challenges <i>Mathukumalli Vidyasagar</i>	15
Predicting Toxicities of Reactive Metabolite–Positive Drug Candidates <i>Amit S. Kalgutkar and Deepak Dalvie</i>	35
The Use of Biomarkers in Human Pharmacology (Phase I) Studies <i>A.F. Cohen, J. Burggraaf, J.M.A. van Gerven, M. Moerland, and G.J. Groeneveld</i>	55
Improving Postapproval Drug Safety Surveillance: Getting Better Information Sooner <i>Sean Hennessy and Brian L. Strom</i>	75
Preemptive Clinical Pharmacogenetics Implementation: Current Programs in Five US Medical Centers <i>Henry M. Dunnenberger, Kristine R. Crews, James M. Hoffman, Kelly E. Caudle, Ulrich Broeckel, Scott C. Howard, Robert J. Hunkler, Teri E. Klein, William E. Evans, and Mary V. Relling</i>	89
A Personalized Medicine Approach for Asian Americans with the Aldehyde Dehydrogenase 2*2 Variant <i>Eric R. Gross, Vanessa O. Zambelli, Bryce A. Small, Julio C.B. Ferreira, Che-Hong Chen, and Daria Mochly-Rosen</i>	107
Pharmacogenomics in Asthma Therapy: Where Are We and Where Do We Go? <i>Heung-Woo Park, Kelan G. Tantisira, and Scott T. Weiss</i>	129

Drug Disposition in Obesity: Toward Evidence-Based Dosing <i>Catherijne A.J. Knibbe, Margreke J.E. Brill, Anne van Rongen, Jeroen Diepstraten, Piet Hein van der Graaf, and Meindert Danhof</i>	149
How Good Is “Evidence” from Clinical Studies of Drug Effects and Why Might Such Evidence Fail in the Prediction of the Clinical Utility of Drugs? <i>Huseyin Naci and John P.A. Ioannidis</i>	169
The Ethics and Economics of Pharmaceutical Pricing <i>Sara Parker-Lue, Michael Santoro, and Greg Koski</i>	191
Brown, Beige, and White: The New Color Code of Fat and Its Pharmacological Implications <i>Alexander Pfeifer and Linda S. Hoffmann</i>	207
Candidate Drug Targets for Prevention or Modification of Epilepsy <i>Nicholas H. Varvel, Jianxiong Jiang, and Raymond Dingledine</i>	229
The Design of Covalent Allosteric Drugs <i>Ruth Nussinov and Chung-Jung Tsai</i>	249
Therapeutic Modulation of Urinary Bladder Function: Multiple Targets at Multiple Levels <i>Martin C. Michel</i>	269
Mineralocorticoids in the Heart and Vasculature: New Insights for Old Hormones <i>Achim Lother, Martin Moser, Christoph Bode, Ross D. Feldman, and Lutz Hein</i>	289
Unraveling the Physiological Complexities of Antibiotic Lethality <i>Daniel J. Dwyer, James J. Collins, and Graham C. Walker</i>	313
Intraclass Differences Among Antihypertensive Drugs <i>R.D. Feldman, Y. Hussain, L.M. Kuyper, F.A. McAlister, R.S. Padwal, and S.W. Tobe</i>	333
Targeting Hsp90/Hsp70-Based Protein Quality Control for Treatment of Adult Onset Neurodegenerative Diseases <i>William B. Pratt, Jason E. Gestwicki, Yoichi Osawa, and Andrew P. Lieberman</i>	353
New Approaches to Inhibiting Platelets and Coagulation <i>Jeremiah P. Depta and Deepak L. Bhatt</i>	373
DREADDs (Designer Receptors Exclusively Activated by Designer Drugs): Chemogenetic Tools with Therapeutic Utility <i>Daniel J. Urban and Bryan L. Roth</i>	399
Learning by Failing: Ideas and Concepts to Tackle γ -Secretases in Alzheimer’s Disease and Beyond <i>Bart De Strooper and Lucía Chávez Gutiérrez</i>	419

Therapeutic Applications of Extracellular Vesicles: Clinical Promise and Open Questions <i>Bence György, Michelle E. Hung, Xandra O. Breakefield, and Joshua N. Leonard</i>	439
Eph Receptors and Ephrins: Therapeutic Opportunities <i>Antonio Barquilla and Elena B. Pasquale</i>	465
Designed Ankyrin Repeat Proteins (DARPs): Binding Proteins for Research, Diagnostics, and Therapy <i>Andreas Plückthun</i>	489
Synthetic Lethal Vulnerabilities of Cancer <i>Ferran Fec de la Cruz, Bianca V. Gapp, and Sebastian M.B. Nijman</i>	513
Calcitonin Gene-Related Peptide (CGRP): A New Target for Migraine <i>Andrew F. Russo</i>	533
Activation and Regulation of Caspase-6 and Its Role in Neurodegenerative Diseases <i>Xiao-Jun Wang, Qin Cao, Yan Zhang, and Xiao-Dong Su</i>	553
Constellation Pharmacology: A New Paradigm for Drug Discovery <i>Russell W. Teichert, Eric W. Schmidt, and Baldomero M. Olivera</i>	573
DNA Methylation and Its Implications and Accessibility for Neuropsychiatric Therapeutics <i>Jeremy J. Day, Andrew J. Kennedy, and J. David Sweatt</i>	591
Targeting Receptor-Mediated Transport for Delivery of Biologics Across the Blood-Brain Barrier <i>Jason M. Lajoie and Eric V. Shusta</i>	613
Novel Targeted Therapies for Eosinophil-Associated Diseases and Allergy <i>Susanne Radonjic-Hoesli, Peter Valent, Amy D. Klion, Michael E. Wechsler, and Hans-Uwe Simon</i>	633

Indexes

Cumulative Index of Contributing Authors, Volumes 51–55	657
Cumulative Index of Article Titles, Volumes 51–55	661

Errata

An online log of corrections to *Annual Review of Pharmacology and Toxicology* articles
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