

REVIEW ARTICLE

DRUG THERAPY

Alastair J.J. Wood, M.D., *Editor*Pharmacogenomics — Drug Disposition,
Drug Targets, and Side Effects

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IT IS WELL RECOGNIZED THAT DIFFERENT PATIENTS RESPOND IN DIFFERENT ways to the same medication. These differences are often greater among members of a population than they are within the same person at different times (or between monozygotic twins).¹ The existence of large population differences with small inpatient variability is consistent with inheritance as a determinant of drug response; it is estimated that genetics can account for 20 to 95 percent of variability in drug disposition and effects.² Although many nongenetic factors influence the effects of medications, including age, organ function, concomitant therapy, drug interactions, and the nature of the disease, there are now numerous examples of cases in which interindividual differences in drug response are due to sequence variants in genes encoding drug-metabolizing enzymes, drug transporters, or drug targets.³⁻⁵ Unlike other factors influencing drug response, inherited determinants generally remain stable throughout a person's lifetime.

Clinical observations of inherited differences in drug effects were first documented in the 1950s,⁶⁻⁹ giving rise to the field of pharmacogenetics, and later pharmacogenomics. Although the two terms are synonymous for all practical purposes, pharmacogenomics uses genome-wide approaches to elucidate the inherited basis of differences between persons in the response to drugs.

More than 1.4 million single-nucleotide polymorphisms were identified in the initial sequencing of the human genome,¹⁰ with over 60,000 of them in the coding region of genes. Some of these single-nucleotide polymorphisms have already been associated with substantial changes in the metabolism or effects of medications, and some are now being used to predict clinical response.^{3-5,11} Because most drug effects are determined by the interplay of several gene products that influence the pharmacokinetics and pharmacodynamics of medications, including inherited differences in drug targets (e.g., receptors) and drug disposition (e.g., metabolizing enzymes and transporters), polygenic determinants of drug effects (Fig. 1) have become increasingly important in pharmacogenomics. In this review, we focus on the therapeutic consequences of inherited differences in drug disposition and drug targets. An accompanying review¹² focuses on the pharmacogenetics of drug metabolism. This review is not meant to be exhaustive; rather, clinically relevant examples are used to illustrate how pharmacogenomics can provide molecular diagnostic methods that improve drug therapy.

GENETIC POLYMORPHISMS INFLUENCING DRUG DISPOSITION

The field of pharmacogenetics began with a focus on drug metabolism,¹² but it has been extended to encompass the full spectrum of drug disposition, including a growing list of transporters that influence drug absorption, distribution, and excretion.^{3-5,13}

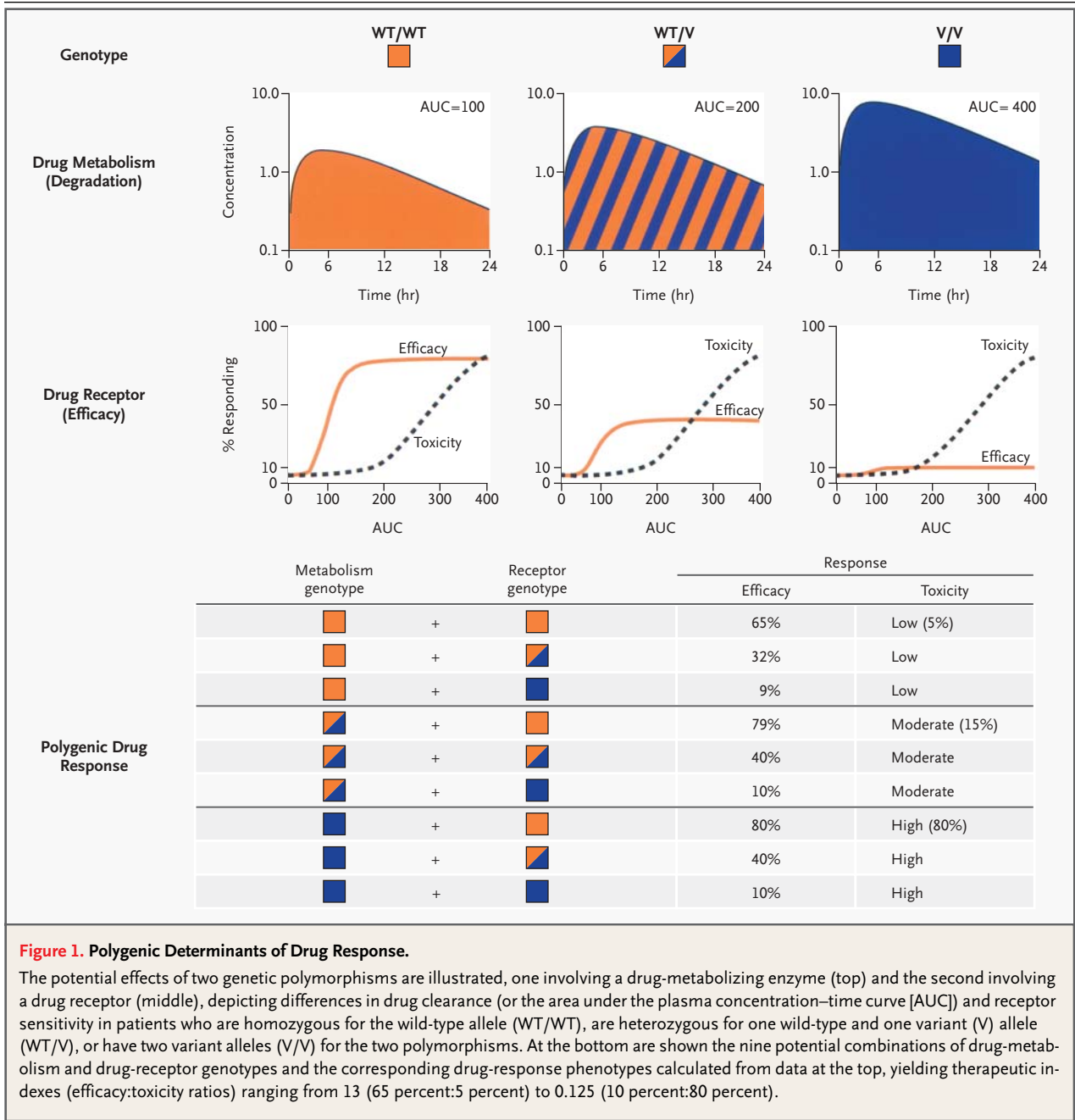


Figure 1. Polygenic Determinants of Drug Response.

The potential effects of two genetic polymorphisms are illustrated, one involving a drug-metabolizing enzyme (top) and the second involving a drug receptor (middle), depicting differences in drug clearance (or the area under the plasma concentration–time curve [AUC]) and receptor sensitivity in patients who are homozygous for the wild-type allele (WT/WT), are heterozygous for one wild-type and one variant (V) allele (WT/V), or have two variant alleles (V/V) for the two polymorphisms. At the bottom are shown the nine potential combinations of drug-metabolism and drug-receptor genotypes and the corresponding drug-response phenotypes calculated from data at the top, yielding therapeutic indexes (efficacy:toxicity ratios) ranging from 13 (65 percent:5 percent) to 0.125 (10 percent:80 percent).

DRUG METABOLISM

There are more than 30 families of drug-metabolizing enzymes in humans,^{3,14} and essentially all have genetic variants, many of which translate into functional changes in the proteins encoded. These monogenic traits are discussed by Weinshilboum.¹² But there is an instructive example of a multigenic effect involving the CYP3A family of P-450 enzymes. About three quarters of whites and half of blacks

have a genetic inability to express functional CYP3A5.¹⁵ The lack of functional CYP3A5 may not be readily evident, because many medications metabolized by CYP3A5 are also metabolized by the universally expressed CYP3A4. For medications that are equally metabolized by both enzymes, the net rate of metabolism is the sum of that due to CYP3A4 and that due to CYP3A5; the existence of this dual pathway partially obscures the clinical effects of ge-

netic polymorphism of CYP3A5 but contributes to the large range of total CYP3A activity in humans (Fig. 2). The CYP3A pathway of drug elimination is further confounded by the presence of single-nucleotide polymorphisms in the CYP3A4 gene that alter the activity of this enzyme for some substrates but not for others.¹⁶ The genetic basis of CYP3A5 deficiency is predominantly a single-nucleotide polymorphism in intron 3 that creates a cryptic splice site causing 131 nucleotides of the intronic sequence to be inserted into the RNA, introducing a termination codon that prematurely truncates the CYP3A5 protein.¹⁵ Although it is now possible to

determine which patients express both functional enzymes (i.e., CYP3A4 and CYP3A5), the clinical importance of these variants for the many drugs metabolized by CYP3A remains unclear.

DRUG TRANSPORTERS

Transport proteins have an important role in regulating the absorption, distribution, and excretion of many medications. Members of the adenosine triphosphate (ATP)-binding cassette family of membrane transporters¹⁷ are among the most extensively studied transporters involved in drug disposition and effects. A member of the ATP-binding cassette family, P-glycoprotein, is encoded by the human ABCB1 gene (also called MDR1). A principal function of P-glycoprotein is the energy-dependent cellular efflux of substrates, including bilirubin, several anticancer drugs, cardiac glycosides, immunosuppressive agents, glucocorticoids, human immunodeficiency virus (HIV) type 1 protease inhibitors, and many other medications (Fig. 3).^{17,21,22} The expression of P-glycoprotein in many normal tissues suggests that it has a role in the excretion of xenobiotics and metabolites into urine, bile, and the intestinal lumen.^{23,24} At the blood-brain barrier, P-glycoprotein in the choroid plexus limits the accumula-

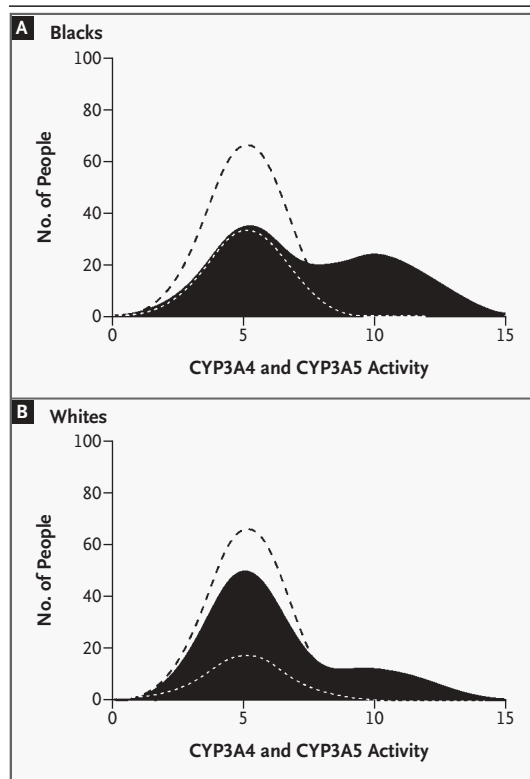
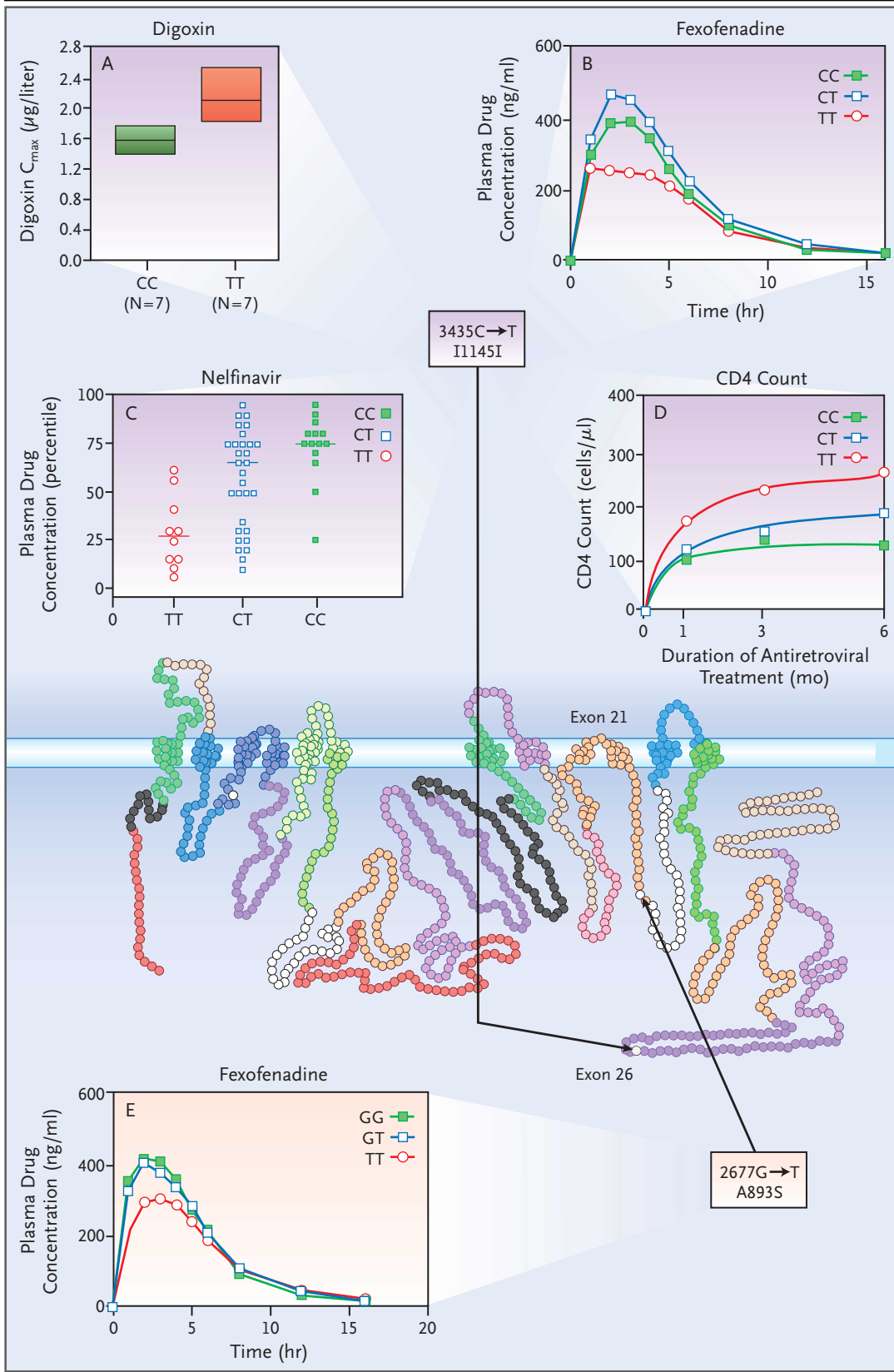


Figure 2. Simulated Activities of Cytochromes P-450 CYP3A4 and CYP3A5 in Blacks and Whites.

The simulated activities of CYP3A4 (black dashed lines) and CYP3A5 (white dashed lines) are shown in blacks (Panel A) and whites (Panel B), assuming a normal distribution and a 10-fold range in activity (shown in arbitrary units) among those expressing functional forms of these enzymes, and further assuming that all patients express CYP3A4, but that only 25 percent of whites and 50 percent of blacks express functional CYP3A5 because of genetic polymorphism. The solid area reflects the combined activity of CYP3A4 and CYP3A5 in the two populations for medications that are metabolized equally by the two enzymes.

Figure 3 (facing page). Functional Consequences of Genetic Polymorphisms in the Human P-Glycoprotein Transporter Gene ABCB1 (or MDR1).

The schematic diagram of the human P-glycoprotein was adapted from Kim et al.,¹⁸ with each circle representing an amino acid and each color a different exon encoding the corresponding amino acids. Two single-nucleotide polymorphisms in the human ABCB1 gene have been associated with altered drug disposition (Panels A, B, C, and E) or altered drug effects (Panel D). The synonymous single-nucleotide polymorphism (a single-nucleotide polymorphism that does not alter the amino acid encoded) in exon 26 (the 3435C→T single-nucleotide polymorphism) has been associated with higher oral bioavailability of digoxin in patients homozygous for the T nucleotide (Panel A [C_{max} denotes maximal concentration])¹⁹ but lower plasma concentrations after oral doses of fexofenadine (Panel B)¹⁸ and nelfinavir (Panel C).²⁰ This single-nucleotide polymorphism has also been linked to better CD4 cell recovery in HIV-infected patients who are treated with nelfinavir and other antiretroviral agents (Panel D).²⁰ The single-nucleotide polymorphism at nucleotide 2677 (G→T) has been associated with lower plasma fexofenadine concentrations in patients homozygous for the T nucleotide at position 2677 (Panel E).¹⁸ The panels have been adapted from Kim et al.,¹⁸ Hoffmeyer et al.,¹⁹ and Fellay et al.²⁰



tion of many drugs in the brain, including digoxin, ivermectin, vinblastine, dexamethasone, cyclosporine, domperidone, and loperamide.²³⁻²⁵ A synonymous single-nucleotide polymorphism (i.e., a single-nucleotide polymorphism that does not alter the amino acid encoded) in exon 26 (3435C→T) has been associated with variable expression of P-glycoprotein in the duodenum; in patients homozygous for the T allele, duodenal expression of P-glycoprotein was less than half that in patients with the CC genotype.¹⁹ CD56+ natural killer cells from subjects homozygous for 3435C demonstrated significantly lower *ex vivo* retention of the P-glycoprotein substrate rhodamine (i.e., higher P-glycoprotein function).²⁶ Digoxin, another P-glycoprotein substrate, has significantly higher bioavailability in subjects with the 3435TT genotype.^{19,27} As is typical for many pharmacogenetic traits, there is considerable racial variation in the frequency of the 3435C→T single-nucleotide polymorphism.²⁸⁻³⁰

The 3435C→T single-nucleotide polymorphism is in linkage disequilibrium with a nonsynonymous single-nucleotide polymorphism (i.e., one causing an amino acid change) in exon 21 (2677G→T, leading to Ala893Ser) that alters P-glycoprotein function.¹⁸ Because these two single-nucleotide polymorphisms travel together, it is unclear whether the 3435C→T polymorphism is of functional importance or is simply linked with the causative polymorphism in exon 21. The 2677G→T single-nucleotide polymorphism has been associated with enhanced P-glycoprotein function *in vitro* and lower plasma fexofenadine concentrations in humans,¹⁸ effects opposite to those reported with digoxin.²⁷

The associations between treatment outcome and genetic variants in *CYP3A4*, *CYP3A5*, *CYP2D6*, *CYP2C19*, the chemokine receptor gene *CCR5*, and *ABCB1* have been examined in HIV-infected patients receiving combination antiretroviral therapy with either a protease inhibitor or a nonnucleoside reverse-transcriptase inhibitor.²⁰ The *ABCB1* 3435C→T polymorphism was associated with significant differences in the plasma pharmacokinetics of nelfinavir (Fig. 3) and efavirenz. Recovery of the CD4 cell count was significantly greater and more rapid in patients with the TT genotype than in patients with either the CT or the CC genotype (Fig. 3). Of many variables evaluated, only the *ABCB1* genotype and the base-line number of HIV RNA copies were significant predictors of CD4 recovery.²⁰ However, the *ABCB1* 2677G→T single-nucleotide polymorphism was not genotyped, so it remains unclear whether the 3435C→T polymorphism is causative or is

simply linked with another polymorphism that is causative.

This example illustrates a common problem in association studies, namely, biologic plausibility. It is not obvious how greater efficacy (CD4 recovery) could be linked to a single-nucleotide polymorphism associated with lower plasma drug concentrations, unless there are specific effects of the *ABCB1* polymorphisms that cause decreased drug efflux from CD4 leukocytes. Overexpression of the gene for another ABC transporter (*ABCC4*, or *MRP4*) confers resistance to some nucleoside antiretroviral agents (e.g., zidovudine).³¹ Despite the uncertainty about the mechanisms involved, the clinical value is that a host genetic marker can predict immune recovery after the initiation of antiretroviral treatment and, if validated, may offer a new strategy in tailoring HIV therapy.

GENETIC POLYMORPHISM OF DRUG TARGETS

Genetic variation in drug targets (e.g., receptors) can have a profound effect on drug efficacy, with over 25 examples already identified (Table 1).³⁻⁵ Sequence variants with a direct effect on response occur in the gene for the β_2 -adrenoreceptor, affecting the response to β_2 -agonists^{43,44}; arachidonate 5-lipoxygenase (*ALOX5*), affecting the response to *ALOX5* inhibitors⁴²; and angiotensin-converting enzyme (*ACE*), affecting the renoprotective actions of *ACE* inhibitors.³² Genetic differences may also have indirect effects on drug response that are unrelated to drug metabolism or transport, such as methylation of the methylguanine methyltransferase (*MGMT*) gene promoter, which alters the response of gliomas to treatment with carmustine.⁶³ The mechanism of this effect is related to a decrease in the efficiency of repair of alkylated DNA in patients with methylated *MGMT*. It is critical to distinguish this target mechanism from genetic polymorphisms in drug-metabolizing enzymes that affect response by altering drug concentrations, such as the thiopurine methyltransferase polymorphism associated with the hematopoietic toxicity of mercaptopurine⁶⁴⁻⁶⁶ and susceptibility to radiation-induced brain tumors.⁶⁷

The β_2 -adrenoreceptor (coded by the *ADRB2* gene) illustrates another link between genetic polymorphisms in drug targets and clinical responses. Genetic polymorphism of the β_2 -adrenoreceptor can alter the process of signal transduction by these receptors.^{43,44} Three single-nucleotide polymor-

Table 1. Genetic Polymorphisms in Drug Target Genes That Can Influence Drug Response.*

Gene or Gene Product	Medication	Drug Effect Associated with Polymorphism
ACE	ACE inhibitors (e.g., enalapril) Fluvastatin	Renoprotective effects, blood-pressure reduction, reduction in left ventricular mass, endothelial function ³²⁻⁴⁰ Lipid changes (e.g., reductions in low-density lipoprotein cholesterol and apolipoprotein B); progression or regression of coronary atherosclerosis ⁴¹
Arachidonate 5-lipoxygenase	Leukotriene inhibitors	Improvement in FEV ₁ ⁴²
β_2 -Adrenergic receptor	β_2 -Agonists (e.g., albuterol)	Bronchodilatation, susceptibility to agonist-induced desensitization, cardiovascular effects ⁴³⁻⁵⁰
Bradykinin B2 receptor	ACE inhibitors	ACE-inhibitor-induced cough ⁵¹
Dopamine receptors (D2, D3, D4)	Antipsychotics (e.g. haloperidol, clozapine)	Antipsychotic response (D2, D3, D4), antipsychotic-induced tardive dyskinesia (D3), antipsychotic-induced acute akathisia (D3) ⁵²⁻⁵⁶
Estrogen receptor- α	Conjugated estrogens Hormone-replacement therapy	Increase in bone mineral density ⁵⁷ Increase in high-density lipoprotein cholesterol ⁵⁸
Glycoprotein IIIa subunit of glycoprotein IIb/IIIa	Aspirin or glycoprotein IIb/IIIa inhibitors	Antiplatelet effect ⁵⁹
Serotonin (5-hydroxytryptamine) transporter	Antidepressants (e.g., clomipramine, fluoxetine, paroxetine)	5-Hydroxytryptamine neurotransmission, antidepressant response ⁶⁰⁻⁶²

* The examples shown are illustrative and not representative of all published studies, which exceed the scope of this review. ACE denotes angiotensin-converting enzyme, and FEV₁ forced expiratory volume in one second.

phisms in ADRB2 have been associated with altered expression, down-regulation, or coupling of the receptor in response to β_2 -adrenoreceptor agonists.⁴³ Single-nucleotide polymorphisms resulting in an Arg-to-Gly amino acid change at codon 16 and a Gln-to-Glu change at codon 27 are relatively common, with allele frequencies of 0.4 to 0.6, and are under intensive investigation for their clinical relevance.

A recent study of agonist-mediated vasodilatation and desensitization⁴⁴ revealed that patients who were homozygous for Arg at ADRB2 codon 16 had nearly complete desensitization after continuous infusion of isoproterenol, with venodilatation decreasing from 44 percent at base line to 8 percent after 90 minutes of infusion (Fig. 4). In contrast, patients homozygous for Gly at codon 16 had no significant change in venodilatation, regardless of their codon 27 status. Polymorphism at codon 27 was also of functional relevance; subjects homozygous for the Glu allele had higher maximal venodilatation in response to isoproterenol than those with the codon 27 Gln genotype, regardless of their codon 16 status (Fig. 4).⁴⁴

These results are generally consistent with those of studies showing that the forced expiratory volume in one second (FEV₁) after a single oral dose of

albuterol was higher by a factor of 6.5 in patients with the Arg/Arg genotype at codon 16 of ADRB2 than in those with the Gly/Gly genotype (Fig. 4).⁴⁸ However, the influence of this genotype was different in patients receiving long-term, regularly scheduled therapy with inhaled β -agonists. Among these patients, those with the Arg/Arg genotype had a gradual decline in the morning peak expiratory flow measured before they had used medication, whereas no change was observed in patients with the Gly/Gly genotype.⁴⁷ In addition, the morning peak expiratory flow deteriorated dramatically after the cessation of therapy in patients with the Arg/Arg genotype, but not in those with the Gly/Gly genotype.⁴⁷ These data suggest that a codon 16 Arg/Arg genotype may identify patients at risk for deleterious or nonbeneficial effects of regularly scheduled therapy with inhaled β -agonists; the data also suggest that these patients may be candidates for alternative schedules of therapy, earlier initiation of anti-inflammatory agents, or both. These findings are also consistent with the aforementioned desensitization of the β_2 -adrenoreceptor in patients with a codon 16 Arg/Arg genotype.⁴⁴

At least 13 distinct single-nucleotide polymorphisms have been identified in ADRB2.⁴⁶ This finding has led to evaluation of the importance of hap-

lotype structure as compared with individual single-nucleotide polymorphisms in determining receptor function and pharmacologic response. Among 77 white, black, Asian, and Hispanic subjects, only 12 distinct haplotypes of the 8192 possible ADRB2 haplotypes were actually observed.⁴⁶ The bronchodilator response to inhaled β -agonist therapy in patients with asthma revealed a stronger association between bronchodilator response and haplotype than between bronchodilator response and any single-nucleotide polymorphism alone.⁴⁶ This is not surprising, because haplotype structure is often a better predictor of phenotypic consequences than are individual polymorphisms. This result suggests that it would be desirable to develop simple but robust molecular methods to determine the haplotype structure of patients.⁶⁸

GENETIC POLYMORPHISMS
WITH INDIRECT EFFECTS
ON DRUG RESPONSE

Polymorphisms in genes encoding proteins that are neither direct targets of medications nor involved in their disposition have been shown to alter the response to treatment in certain situations (Table 2). For example, inherited differences in coagulation factors can predispose women taking oral contraceptives to deep-vein or cerebral-vein thrombosis,⁸⁰ whereas polymorphisms in the gene for the cholesterol ester transfer protein have been linked to the progression of atherosclerosis with pravastatin therapy.⁷⁵

Genetic variation in cellular ion transporters can also have an indirect role in predisposing patients to toxic effects of drugs. For example, patients with variant alleles for sodium or potassium transporters may have substantial morbidity or mortality resulting from drug-induced long-QT syndrome. A mutation in *KCNE2*, the gene for an integral membrane subunit that assembles with *HERG* to form I_{Kr} potassium channels, was identified in a patient who had cardiac arrhythmia after receiving clarithromycin.⁷⁶ Additional *KCNE2* variants have been associated with the development of a very long QT interval after therapy with trimethoprim-sulfamethoxazole, with sulfamethoxazole inhibiting potassium channels encoded by the *KCNE2* (8T→A) variant.⁷⁷ Because *KCNE2* variants occur in about 1.6 percent of the population and their effect on drug actions can cause death, they are excellent candidates for polygenic strategies to prevent serious drug-induced toxic effects.

Genetic polymorphism in the apolipoprotein E (*APOE*) gene appears to have a role in predicting responses to therapy for Alzheimer's disease and to lipid-lowering drugs.^{70,71,82,83} There are numerous allelic variants of the human *APOE* gene (e.g., *APOE* ϵ 3, *APOE* ϵ 4, *APOE* ϵ 5, etc.), which contain one or more single-nucleotide polymorphisms that alter the amino acid sequence of the encoded protein (e.g., apolipoprotein ϵ 4 has a Cys112Arg change). In a study of treatment of Alzheimer's disease with tacrine, 83 percent of the patients without any *APOE* ϵ 4 allele showed improvement in total response and cognitive response after 30 weeks, as compared with 40 percent of patients with at least one *APOE* ϵ 4 allele.⁷² However, the greatest individual improvement in this study was seen in a patient with a single *APOE* ϵ 4 allele, the unfavorable genotype, illustrating that a single gene will not always predict the response to a given treatment.⁷² Follow-up studies indicate that the interaction between tacrine treatment and *APOE* genotype was strongest for women, again suggesting that many genes are involved in determining the efficacy of a treatment.⁸⁴

The molecular basis for an association between apolipoprotein genotype and tacrine efficacy has not been elucidated, but it has been postulated that the *APOE* ϵ 4 genotype may have an effect on cholinergic dysfunction in Alzheimer's disease that cannot be consistently overcome by therapy with acetylcholinesterase inhibitors such as tacrine. A randomized, placebo-controlled study of the noradrenergic vasopressinergic agonist S12024 in patients with Alzheimer's disease found the greatest protection of cognition in patients with the *APOE* ϵ 4 genotype.⁸⁵ Confirmation of these results may offer an approach to the selection of initial therapy for Alzheimer's disease, with S12024 or similar medications being recommended for patients carrying an *APOE* ϵ 4 allele.

Both phenotypic analysis and genotypic analysis of the *APOE* polymorphism have shown an asso-

Figure 4 (facing page). Functional Consequence of Genetic Polymorphisms in the β_2 -Adrenoreceptor (Coded by the *ADRB2* Gene) at Codons 16 and 27.

A homozygous Glu genotype at codon 27 is associated with greater venodilatation after the administration of isoproterenol (Panel A).⁴⁴ A homozygous Arg genotype at codon 16 is associated with greater airway response to oral albuterol (Panel B)⁴⁸ and greater desensitization to isoproterenol (Panel C).⁴⁴ FEV₁ denotes forced expiratory volume in one second.

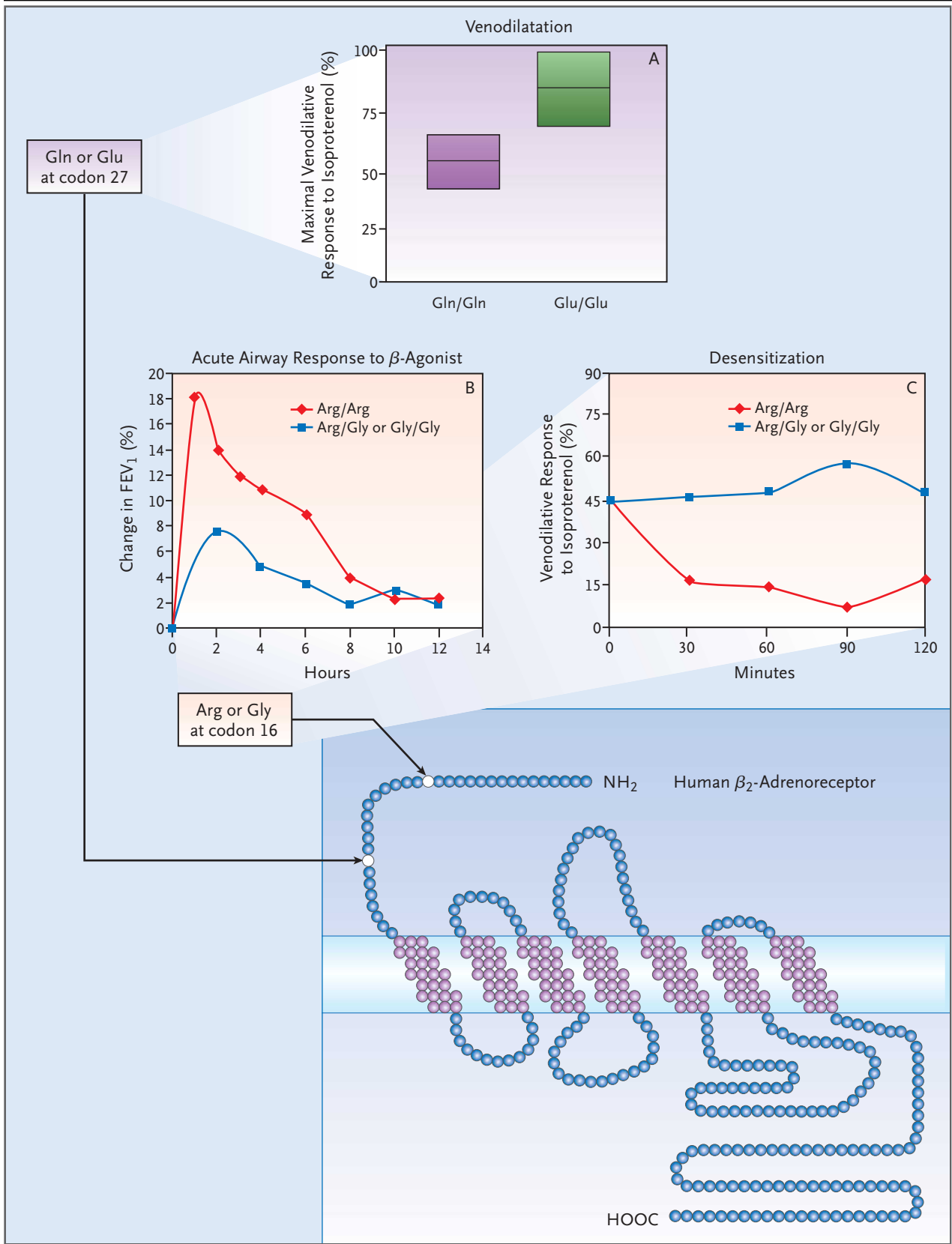


Table 2. Genetic Polymorphisms in Disease-Modifying or Treatment-Modifying Genes That Can Influence Drug Response.*

Gene or Gene Product	Disease or Response Association	Medication	Influence of Polymorphism on Drug Effect or Toxicity
Adducin	Hypertension	Diuretics	Myocardial infarction or strokes ⁶⁹
Apolipoprotein E (APOE)	Progression of atherosclerosis, ischemic cardiovascular events	Statins (e.g., simvastatin)	Enhanced survival ^{70,71}
Apolipoprotein E (APOE)	Alzheimer's disease	Tacrine	Clinical improvement ⁷²
HLA	Toxicity	Abacavir	Hypersensitivity reaction ^{73,74}
Cholesterol ester transfer protein (CETP)	Progression of atherosclerosis	Statins (e.g., pravastatin)	Slowing of progression of atherosclerosis by pravastatin ⁷⁵
Ion channels (HERG, KvLQT1, Mink, MiRP1)	Congenital long-QT syndrome	Erythromycin, terfenadine, cisapride, clarithromycin, quinidine	Increased risk of drug-induced torsade de pointes ⁷⁶⁻⁷⁸
Methylguanine methyltransferase (MGMT)	Glioma	Carmustine	Response of glioma to carmustine ⁶³
<i>Parkin</i>	Parkinson's disease	Levodopa	Clinical improvement and levodopa-induced dyskinesias ⁷⁹
Prothrombin and factor V	Deep-vein thrombosis and cerebral-vein thrombosis	Oral contraceptives	Increased risk of deep-vein and cerebral-vein thrombosis with oral contraceptives ⁸⁰
Stromelysin-1	Atherosclerosis progression	Statins (e.g., pravastatin)	Reduction in cardiovascular events by pravastatin (death, myocardial infarction, stroke, angina, and others); reduction in risk of repeated angioplasty ⁸¹

* The examples shown are illustrative and not representative of all published studies, which exceed the scope of this review.

ciation between APOE genotype and the response to lipid-lowering medications.^{82,86-89} In most studies, patients with an APOE $\epsilon 2$ allele had the greatest diminution of low-density lipoprotein cholesterol after drug therapy. The decrease was greatest for those with APOE $\epsilon 2$, followed by APOE $\epsilon 3$ and then APOE $\epsilon 4$. This result was observed after treatment with a diverse range of lipid-lowering agents, including probucol, gemfibrozil, and many different 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors (statins).⁸³ However, a significant effect of APOE genotype on the response to lipid-lowering agents has not been observed in all studies.⁸³

In addition, although the APOE4 allele was associated with less reduction in total and low-density lipoprotein cholesterol and a smaller increase in high-density lipoprotein cholesterol after fluvastatin therapy, there was no apparent influence of genotype on the progression of coronary artery disease or the incidence of clinical events.⁸⁸ Thus, prospective clinical evaluations with robust clinical end points and sufficient sample sizes are needed to define better the usefulness of the APOE genotype in selecting the treatment of hyperlipidemia and cardiovascular disease. The potential usefulness of

the APOE genotype in predicting treatment response must be balanced by the concern that it could be used by insurance companies, health systems, and others to identify those at high risk for Alzheimer's disease, coronary artery disease, and possibly other illnesses.⁸²

MOLECULAR DIAGNOSTIC METHODS FOR OPTIMIZING DRUG THERAPY

The potential is enormous for pharmacogenomics to yield a powerful set of molecular diagnostic methods that will become routine tools with which clinicians will select medications and drug doses for individual patients. A patient's genotype needs to be determined only once for any given gene, because except for rare somatic mutations, it does not change. Genotyping methods are improving so rapidly that it will soon be simple to test for thousands of single-nucleotide polymorphisms in one assay. It may be possible to collect a single blood sample from a patient, submit a small aliquot for analysis of a panel of genotypes (e.g., 20,000 single-nucleotide polymorphisms in 5000 genes), and test for those that are important determinants of drug disposition

and effects. In our opinion, genotyping results will be of greatest clinical value if they are reported and interpreted according to the patient's diagnosis and recommended treatment options.

CHALLENGES FOR THE FUTURE

There are a number of critical issues that must be considered as strategies are developed to elucidate the inherited determinants of drug effects. A formidable one is that the inherited component of the response to drugs is often polygenic (Fig. 1). Approaches for elucidating polygenic determinants of drug response include the use of anonymous single-nucleotide polymorphism maps to perform genome-wide searches for polymorphisms associated with drug effects, and candidate-gene strategies based on existing knowledge of a medication's mechanisms of action and pathways of metabolism and disposition. Both these strategies have potential value and limitations, as shown in previous reviews.^{5,90,91} However, the candidate-gene strategy has the advantage of focusing resources on a manageable number of genes and polymorphisms that are likely to be important, and it has produced encouraging results in a number of studies.^{20,52} The limitations of this approach are the incompleteness of knowledge of a medication's pharmacokinetics and mechanisms of action. Gene-expression profiling^{92,93} and proteomic studies⁹⁴ are evolving strategies for identifying genes that may influence drug response.

One of the most important challenges in defining pharmacogenetic traits is the need for well-characterized patients who have been uniformly treated and systematically evaluated to make it possible to quantitate drug response objectively. To this end, the norm should be to obtain genomic DNA from all patients enrolled in clinical drug trials, along with appropriate consent to permit pharmacogenetic studies. Because of marked population heterogeneity, a specific genotype may be important in determining the effects of a medication for one population or disease but not for another; therefore, pharmacogenomic relations must be validated for each therapeutic indication and in different racial and ethnic groups. Remaining cognizant of these caveats will help ensure accurate elucidation of genetic determinants of drug response and facilitate the translation of pharmacogenomics into widespread clinical practice.

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REFERENCES

- Vesell ES. Pharmacogenetic perspectives gained from twin and family studies. *Pharmacol Ther* 1989;41:535-52.
- Kalow W, Tang BK, Endrenyi I. Hypothesis: comparisons of inter- and intra-individual variations can substitute for twin studies in drug research. *Pharmacogenetics* 1998;8:283-9.
- Evans WE, Relling MV. Pharmacogenomics: translating functional genomics into rational therapeutics. *Science* 1999;286:487-91.
- Evans WE, Johnson JA. Pharmacogenomics: the inherited basis for interindividual differences in drug response. *Annu Rev Genomics Hum Genet* 2001;2:9-39.
- McLeod HL, Evans WE. Pharmacogenomics: unlocking the human genome for better drug therapy. *Annu Rev Pharmacol Toxicol* 2001;41:101-21.
- Kalow W. Familial incidence of low pseudocholinesterase level. *Lancet* 1956;2:576.
- Carson PE, Flanagan CL, Ickes CE, Alving AS. Enzymatic deficiency in primaquine-sensitive erythrocytes. *Science* 1956;124:484-5.
- Hughes HB, Biehl JP, Jones AP, Schmidt LH. Metabolism of isoniazid in man as related to the occurrence of peripheral neuritis. *Am Rev Tuberc* 1954;70:266-73.
- Evans DAP, Manley KA, McKusick VA. Genetic control of isoniazid metabolism in man. *Br Med J* 1960;2:485-91.
- Sachidanandam R, Weissman D, Schmidt SC, et al. A map of human genome sequence variation containing 1.42 million single nucleotide polymorphisms. *Nature* 2001;409:928-33.
- Yates CR, Krynetski EY, Loennechen T, et al. Molecular diagnosis of thiopurine S-methyltransferase deficiency: genetic basis for azathioprine and mercaptopurine intolerance. *Ann Intern Med* 1997;126:608-14.
- Weinsilboum R. Inheritance and drug response. *N Engl J Med* 2003;348:529-37.
- Meyer UA. Pharmacogenetics and adverse drug reactions. *Lancet* 2000;356:1667-71.
- Ingelman-Sundberg M, Oscarson M, McLellan RA. Polymorphic human cytochrome P450 enzymes: an opportunity for individualized drug treatment. *Trends Pharmacol Sci* 1999;20:342-9.
- Kuehl P, Zhang J, Lin Y, et al. Sequence diversity in CYP3A promoters and characterization of the genetic basis for polymorphic CYP3A5 expression. *Nat Genet* 2001;27:383-91.
- Sata F, Sapone A, Elizondo G, et al. CYP3A4 allelic variants with amino acid substitutions in exons 7 and 12: evidence for an allelic variant with altered catalytic activity. *Clin Pharmacol Ther* 2000;67:48-56.
- Borst P, Evers R, Kool M, Wijnholds J. A family of drug transporters: the multidrug resistance-associated proteins. *J Natl Cancer Inst* 2000;92:1295-302.
- Kim RB, Leake BF, Choo EF, et al. Identification of functionally variant MDR1 alleles

- among European Americans and African Americans. *Clin Pharmacol Ther* 2001;70:189-99.
19. Hoffmeyer S, Burk O, von Richter O, et al. Functional polymorphisms of the human multidrug-resistance gene: multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity in vivo. *Proc Natl Acad Sci U S A* 2000;97:3473-8.
20. Fellay J, Marzolini C, Meaden ER, et al. Response to antiretroviral treatment in HIV-1-infected individuals with allelic variants of the multidrug resistance transporter 1: a pharmacogenetics study. *Lancet* 2002;359:30-6.
21. Choo EF, Leake B, Wandel C, et al. Pharmacological inhibition of P-glycoprotein transport enhances the distribution of HIV-1 protease inhibitors into brain and testes. *Drug Metab Dispos* 2000;28:655-60.
22. Brinkmann U, Roots I, Eichelbaum M. Pharmacogenetics of the human drug-transporter gene MDR1: impact of polymorphisms on pharmacotherapy. *Drug Discov Today* 2001;6:835-9.
23. Rao VV, Dahlheimer JL, Bardgett ME, et al. Choroid plexus epithelial expression of MDR1 P glycoprotein and multidrug resistance-associated protein contribute to the blood-cerebrospinal-fluid drug-permeability barrier. *Proc Natl Acad Sci U S A* 1999;96:3900-5.
24. Thiebaut F, Tsuruo T, Hamada H, Gottesman MM, Pastan I, Willingham MC. Cellular localization of the multidrug-resistance gene product P-glycoprotein in normal human tissues. *Proc Natl Acad Sci U S A* 1987;84:7735-8.
25. Schinkel AH, Wagenaar E, Mol CA, van Deemter L. P-glycoprotein in the blood-brain barrier of mice influences the brain penetration and pharmacological activity of many drugs. *J Clin Invest* 1996;97:2517-24.
26. Hitzl M, Drescher S, van der Kuip H, et al. The C3435T mutation in the human MDR1 gene is associated with altered efflux of the P-glycoprotein substrate rhodamine 123 from CD56+ natural killer cells. *Pharmacogenetics* 2001;11:293-8.
27. Sakaeda T, Nakamura T, Horinouchi M, et al. MDR1 genotype-related pharmacokinetics of digoxin after single oral administration in healthy Japanese subjects. *Pharm Res* 2001;18:1400-4.
28. Ameyaw MM, Regateiro F, Li T, et al. MDR1 pharmacogenetics: frequency of the C3435T mutation in exon 26 is significantly influenced by ethnicity. *Pharmacogenetics* 2001;11:217-21.
29. McLeod H. Pharmacokinetic differences between ethnic groups. *Lancet* 2002;359:78.
30. Schaeffeler E, Eichelbaum M, Brinkmann U, et al. Frequency of C3435T polymorphism of MDR1 gene in African people. *Lancet* 2001;358:383-4.
31. Schuetz JD, Connelly MC, Sun D, et al. MRP4: a previously unidentified factor in resistance to nucleoside-based antiviral drugs. *Nat Med* 1999;5:1048-51.
32. Jacobsen P, Rossing K, Rossing P, et al. Angiotensin converting enzyme gene polymorphism and ACE inhibition in diabetic nephropathy. *Kidney Int* 1998;53:1002-6.
33. Kohno M, Yokokawa K, Minami M, et al. Association between angiotensin-converting enzyme gene polymorphisms and regression of left ventricular hypertrophy in patients treated with angiotensin-converting enzyme inhibitors. *Am J Med* 1999;106:544-9.
34. Ohmichi N, Iwai N, Uchida Y, Shichiri G, Nakamura Y, Kinoshita M. Relationship between the response to the angiotensin converting enzyme inhibitor imidapril and the angiotensin converting enzyme genotype. *Am J Hypertens* 1997;10:951-5.
35. Okamura A, Ohishi M, Rakugi H, et al. Pharmacogenetic analysis of the effect of angiotensin-converting enzyme inhibitor on restenosis after percutaneous transluminal coronary angioplasty. *Angiology* 1999;50:811-22.
36. Penno G, Chaturvedi N, Talmud PJ, et al. Effect of angiotensin-converting enzyme (ACE) gene polymorphism on progression of renal disease and the influence of ACE inhibition in IDDM patients: findings from the EUCLID Randomized Controlled Trial: EURODIAB Controlled Trial of Lisinopril in IDDM. *Diabetes* 1998;47:1507-11.
37. Perna A, Ruggenti P, Testa A, et al. ACE genotype and ACE inhibitors induced renoprotection in chronic proteinuric nephropathies. *Kidney Int* 2000;57:274-81.
38. Prasad A, Narayanan S, Husain S, et al. Insertion-deletion polymorphism of the ACE gene modulates reversibility of endothelial dysfunction with ACE inhibition. *Circulation* 2000;102:35-41.
39. Sasaki M, Oki T, Iuchi A, et al. Relationship between the angiotensin converting enzyme gene polymorphism and the effects of enalapril on left ventricular hypertrophy and impaired diastolic filling in essential hypertension: M-mode and pulsed Doppler echocardiographic studies. *J Hypertens* 1996;14:1403-8.
40. Stavroulakis GA, Makris TK, Krespi PG, et al. Predicting response to chronic antihypertensive treatment with fosinopril: the role of angiotensin-converting enzyme gene polymorphism. *Cardiovasc Drugs Ther* 2000;14:427-32.
41. Marian AJ, Safavi F, Ferlic L, Dunn JK, Gotto AM, Ballantyne CM. Interactions between angiotensin-I converting enzyme insertion/deletion polymorphism and response of plasma lipids and coronary atherosclerosis to treatment with fluvastatin: the Lipoprotein and Coronary Atherosclerosis Study. *J Am Coll Cardiol* 2000;35:89-95.
42. Drazen JM, Yandava CN, Dube L, et al. Pharmacogenetic association between ALOX5 promoter genotype and the re-sponse to anti-asthma treatment. *Nat Genet* 1999;22:168-70.
43. Liggett SB. Beta(2)-adrenergic receptor pharmacogenetics. *Am J Respir Crit Care Med* 2000;161:S197-S201.
44. Dishy V, Sofowora GG, Xie H-G, et al. The effect of common polymorphisms of the β_2 -adrenergic receptor on agonist-mediated vascular desensitization. *N Engl J Med* 2001;345:1030-5.
45. Cockcroft JR, Gazis AG, Cross DJ, et al. Beta(2)-adrenoceptor polymorphism determines vascular reactivity in humans. *Hypertension* 2000;36:371-5.
46. Drysdale CM, McGraw DW, Stack CB, et al. Complex promoter and coding region beta 2-adrenergic receptor haplotypes alter receptor expression and predict in vivo responsiveness. *Proc Natl Acad Sci U S A* 2000;97:10483-8.
47. Israel E, Drazen JM, Liggett SB, et al. Effect of polymorphism of the beta(2)-adrenergic receptor on response to regular use of albuterol in asthma. *Int Arch Allergy Immunol* 2001;124:183-6.
48. Lima JJ, Thomason DB, Mohamed MH, Eberle LV, Self TH, Johnson JA. Impact of genetic polymorphisms of the beta2-adrenergic receptor on albuterol bronchodilator pharmacodynamics. *Clin Pharmacol Ther* 1999;65:519-25.
49. Martinez FD, Graves PE, Baldini M, Solomon S, Erickson R. Association between genetic polymorphisms of the beta2-adrenoceptor and response to albuterol in children with and without a history of wheezing. *J Clin Invest* 1997;100:3184-8.
50. Tan S, Hall IP, Dewar J, Dow E, Lipworth B. Association between beta 2-adrenoceptor polymorphism and susceptibility to bronchodilator desensitization in moderately severe stable asthmatics. *Lancet* 1997;350:995-9.
51. Mukae S, Aoki S, Itoh S, Iwata T, Ueda H, Katagiri T. Bradykinin B(2) receptor gene polymorphism is associated with angiotensin-converting enzyme inhibitor-related cough. *Hypertension* 2000;36:127-31.
52. Arranz MJ, Munro J, Birkett J, et al. Pharmacogenetic prediction of clozapine response. *Lancet* 2000;355:1615-6.
53. Basile VS, Masellis M, Badri F, et al. Association of the MscI polymorphism of the dopamine D3 receptor gene with tardive dyskinesia in schizophrenia. *Neuropsychopharmacology* 1999;21:17-27.
54. Eichhammer P, Albus M, Borrmann-Hassenbach M, et al. Association of dopamine D3-receptor gene variants with neuroleptic induced akathisia in schizophrenic patients: a generalization of Steen's study on DRD3 and tardive dyskinesia. *Am J Med Genet* 2000;96:187-91.
55. Hwu HG, Hong CJ, Lee YL, Lee PC, Lee SF. Dopamine D4 receptor gene polymorphisms and neuroleptic response in schizophrenia. *Biol Psychiatry* 1998;44:483-7.
56. Kaiser R, Konneker M, Henneken M, et al. Dopamine D4 receptor 48-bp repeat polymorphism: no association with response to antipsychotic treatment, but association with catatonic schizophrenia. *Mol Psychiatry* 2000;5:418-24.
57. Ongphiphadhanakul B, Chanprasertyothin S, Payatikul P, et al. Oestrogen-

- receptor-alpha gene polymorphism affects response in bone mineral density to oestrogen in post-menopausal women. *Clin Endocrinol (Oxf)* 2000;52:581-5.
58. Herrington DM, Howard TD, Hawkins GA, et al. Estrogen-receptor polymorphisms and effects of estrogen replacement on high-density lipoprotein cholesterol in women with coronary disease. *N Engl J Med* 2002;346:967-74.
59. Michelson AD, Furman MI, Goldschmidt-Clermont P, et al. Platelet GP IIIa P1(A) polymorphisms display different sensitivities to agonists. *Circulation* 2000;101:1013-8.
60. Kim DK, Lim SW, Lee S, et al. Serotonin transporter gene polymorphism and antidepressant response. *Neuroreport* 2000;11:215-9.
61. Smeraldi E, Zanardi R, Benedetti F, Di Bella D, Perez J, Catalano M. Polymorphism within the promoter of the serotonin transporter gene and antidepressant efficacy of fluvoxamine. *Mol Psychiatry* 1998;3:508-11.
62. Whale R, Quested DJ, Laver D, Harrison PJ, Cowen PJ. Serotonin transporter (5-HTT) promoter genotype may influence the prolactin response to clomipramine. *Psychopharmacology (Berl)* 2000;150:120-2.
63. Esteller M, Garcia-Foncillas J, Andion E, et al. Inactivation of the DNA-repair gene MGMT and the clinical response of gliomas to alkylating agents. *N Engl J Med* 2000;343:1350-4. [Erratum, *N Engl J Med* 2000;343:1740.]
64. Evans WE, Hon YY, Bomgaars L, et al. Preponderance of thiopurine S-methyltransferase deficiency and heterozygosity among patients intolerant to mercaptopurine or azathioprine. *J Clin Oncol* 2001;19:2293-301.
65. Black AJ, McLeod HL, Capell HA, et al. Thiopurine methyltransferase genotype predicts therapy-limiting severe toxicity from azathioprine. *Ann Intern Med* 1998;129:716-8.
66. Relling MV, Hancock ML, Rivera GK, et al. Mercaptopurine therapy intolerance and heterozygosity at the thiopurine S-methyltransferase gene locus. *J Natl Cancer Inst* 1999;91:2001-8.
67. Relling MV, Rubnitz JE, Rivera GK, et al. High incidence of secondary brain tumours after radiotherapy and antimetabolites. *Lancet* 1999;354:34-9.
68. McDonald OG, Krynetski EY, Evans WE. Molecular haplotyping of genomic DNA for multiple single-nucleotide polymorphisms located kilobases apart using long-range polymerase chain reaction and intramolecular ligation. *Pharmacogenetics* 2002;12:93-9.
69. Psaty BM, Smith NL, Heckbert SR, et al. Diuretic therapy, the α -adducin gene variation, and the risk of myocardial infarction or stroke in persons with treated hypertension. *JAMA* 2002;287:1680-9.
70. Gerdes LU, Gerdes C, Kervinen K, et al. The apolipoprotein epsilon4 allele determines prognosis and the effect on prognosis of simvastatin in survivors of myocardial infarction: a substudy of the Scandinavian Simvastatin Survival Study. *Circulation* 2000;101:1366-71.
71. Ordovas JM, Lopez-Miranda J, Perez-Jimenez F, et al. Effect of apolipoprotein E and A-IV phenotypes on the low density lipoprotein response to HMG CoA reductase inhibitor therapy. *Atherosclerosis* 1995;113:157-66.
72. Poirier J, Delisle MC, Quirion R, et al. Apolipoprotein E4 allele as a predictor of cholinergic deficits and treatment outcome in Alzheimer disease. *Proc Natl Acad Sci U S A* 1995;92:12260-4.
73. Mallal S, Nolan D, Witt C, et al. Association between presence of HLA-B*5701, HLA-DR7, and HLA-DQ3 and hypersensitivity to HIV-1 reverse-transcriptase inhibitor abacavir. *Lancet* 2002;359:727-32.
74. Hetherington S, Hughes AR, Mosteller M, et al. Genetic variations in HLA-B region and hypersensitivity reaction to abacavir. *Lancet* 2002;359:1121-2.
75. Kuivenhoven JA, Jukema JW, Zwiderman AH, et al. The role of a common variant of the cholesteryl ester transfer protein gene in the progression of coronary atherosclerosis. *N Engl J Med* 1998;338:86-93.
76. Abbott GW, Sesti F, Splawski I, et al. MiRP1 forms IKr potassium channels with HERG and is associated with cardiac arrhythmia. *Cell* 1999;97:175-87.
77. Sesti F, Abbott GW, Wei J, et al. A common polymorphism associated with antibiotic-induced cardiac arrhythmia. *Proc Natl Acad Sci U S A* 2000;97:10613-8.
78. Napolitano C, Schwartz PJ, Brown AM, et al. Evidence for a cardiac ion channel mutation underlying drug-induced QT prolongation and life-threatening arrhythmias. *J Cardiovasc Electrophysiol* 2000;11:691-6.
79. Lücking CB, Dürr A, Bonifati V, et al. Association between early-onset Parkinson's disease and mutations in the parkin gene. *N Engl J Med* 2000;342:1560-7.
80. Martinelli I, Sacchi E, Landi G, Taioli E, Duca F, Mannucci PM. High risk of cerebral-vein thrombosis in carriers of a prothrombin-gene mutation and in users of oral contraceptives. *N Engl J Med* 1998;338:1793-7.
81. de Maat MP, Jukema JW, Ye S, et al. Effect of the stromelysin-1 promoter on efficacy of pravastatin in coronary atherosclerosis and restenosis. *Am J Cardiol* 1999;83:852-6.
82. Issa AM, Keyserlingk EW. Apolipoprotein E genotyping for pharmacogenetic purposes in Alzheimer's disease: emerging ethical issues. *Can J Psychiatry* 2000;45:917-22.
83. Siest G, Bertrand P, Herbeth B, et al. Apolipoprotein E polymorphisms and concentration in chronic diseases and drug responses. *Clin Chem Lab Med* 2000;38:841-52.
84. Farlow MR, Lahiri DK, Poirier J, Davignon J, Schneider L, Hui SL. Treatment outcome of tacrine therapy depends on apolipoprotein genotype and gender of the subjects with Alzheimer's disease. *Neurology* 1998;50:669-77.
85. Richard F, Helbecque N, Neuman E, Guez D, Levy R, Amouyel P. APOE genotyping and response to drug treatment in Alzheimer's disease. *Lancet* 1997;349:539.
86. Nestruck AC, Bouthillier D, Sing CF, Davignon J. Apolipoprotein E polymorphism and plasma cholesterol response to probucol. *Metabolism* 1987;36:743-7.
87. Pedro-Botet J, Schaefer EJ, Bakker-Arkema RG, et al. Apolipoprotein E genotype affects plasma lipid response to atorvastatin in a gender specific manner. *Atherosclerosis* 2001;158:183-93.
88. Watanabe J, Kobayashi K, Umeda F, et al. Apolipoprotein E polymorphism affects the response to pravastatin on plasma apolipoproteins in diabetic patients. *Diabetes Res Clin Pract* 1993;20:21-7.
89. Ballantyne CM, Herd JA, Stein EA, et al. Apolipoprotein E genotypes and response of plasma lipids and progression-regression of coronary atherosclerosis to lipid-lowering drug therapy. *J Am Coll Cardiol* 2000;36:1572-8.
90. Cargill M, Daley GQ. Mining for SNPs: putting the common variants-common disease hypothesis to the test. *Pharmacogenomics* 2000;1:27-37.
91. Sham P. Shifting paradigms in gene-mapping methodology for complex traits. *Pharmacogenomics* 2001;2:195-202.
92. Staunton JE, Slonim DK, Collier HA, et al. Chemosensitivity prediction by transcriptional profiling. *Proc Natl Acad Sci U S A* 2001;98:10787-92.
93. Yeoh EJ, Ross ME, Shurtleff SA, et al. Classification, subtype discovery, and prediction of outcome in pediatric acute lymphoblastic leukemia by gene expression profiling. *Cancer Cell* 2002;1:133-43.
94. Liotta LA, Kohn EC, Petricoin EF. Clinical proteomics: personalized molecular medicine. *JAMA* 2001;286:2211-4.

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