PAPER

Pharmacogenomics: marshalling the human genome to individualise drug therapy

W E Evans

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Pharmacogenomics aims to identify the inherited basis for interindividual differences in drug response, and translate this to molecular diagnostics that can be used to individualise drug therapy. This review uses a number of published examples of inherited differences in drug metabolising enzymes, drug transporters, and drug targets (for example, receptors) to illustrate the potential importance of inheritance in determining the efficacy and toxicity of medications in humans. It seems that this field is at the early stages of developing a powerful set of molecular diagnostics that will have profound utility in optimising drug therapy for individual patients.

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Pharmacogenomics is a rapidly emerging field that aims to elucidate the genetic basis for interindividual differences in drug response, using genome wide approaches to identify genetic polymorphisms that govern an individual's response to specific drugs.[1–4] As described in the initial reports from the human genome project,[5] there are over 1.4 million single nucleotide polymorphisms (SNPs) in the human genome, with over 60 000 of these residing in the coding region of human genes, and the number of SNPs will grow as more humans are studied. Some of these SNPs have already been associated with significant changes in the metabolism or effects of commonly used drugs and are beginning to make their way into clinical medicine as molecular diagnostics.[2–4] For some genetic polymorphisms (for example, thiopurine S-methyltransferase (TPMT), cytochrome P4502D6), monogenic traits have a marked effect on drug disposition (for example, pharmacokinetic changes attributable to aberrant drug metabolism), and people who inherit the enzyme deficiency must be treated with substantially different doses of some affected drugs (for example, 5%–10% of the standard mercaptopurine or azathioprine dose in patients inheriting two mutant TPMT alleles).[6–8] Likewise, polymorphisms in drug targets (for example, β_2 adrenoceptor, 5-lipoxygenase) have been shown to change the sensitivity of patients to treatment with medications that interact with these targets (for example, β agonists, zileuton), changing the pharmacodynamics of drug response.[9 10] Because most drug effects are determined by the interplay of several gene products that govern the pharmacokinetics and pharmacodynamics of medications, pharmacogenomics is increasingly focused on elucidating polygenic determinants of drug effects (fig 1).

The potential importance of pharmacogenetics has been recognised for many years; clinical observations of inherited differences in drug effects were first reported in the 1950s[11–14] giving rise to the field of "pharmacogenetics", which has now been embraced by a broader spectrum of academia and industry, giving birth to "pharmacogenomics". The two terms are commonly used interchangeably to describe genetic determinants of drug disposition and response. There are now numerous examples establishing that interindividual differences can be attributed, at least in part, to polymorphisms in genes encoding drug metabolising enzymes, drug transporters and/or drug targets (for example, receptors, enzymes).[1–4] While it is clear that many non-genetic factors influence the effects of medications, including a person's age, race, sex, organ function, concomitant therapy, disease severity, and drug interactions, inherited determinants of drug disposition and effects remain stable for a person's lifetime and can have marked effects, independent of the non-genetic factors.

The human genes involved in many pharmacogenetic traits have now been isolated, their molecular mechanisms elucidated, and their clinical importance more clearly defined (reviewed in references[1–4]). This review provides a number of examples of genetic polymorphisms that determine a person's response to drugs, and how this can be translated to clinical practice via molecular diagnostics (genotyping) to guide the selection of medications and drug doses that are optimal for the individual patient.

POLYMORPHISMS IN GENES INFLUENCING DRUG DISPOSITION
While pharmacogenetics began with a focus on drug metabolism, it has now been extended to all aspects of drug disposition, including a growing list of drug transporters that influence drug absorption, distribution, and excretion (table 1).[1–4]
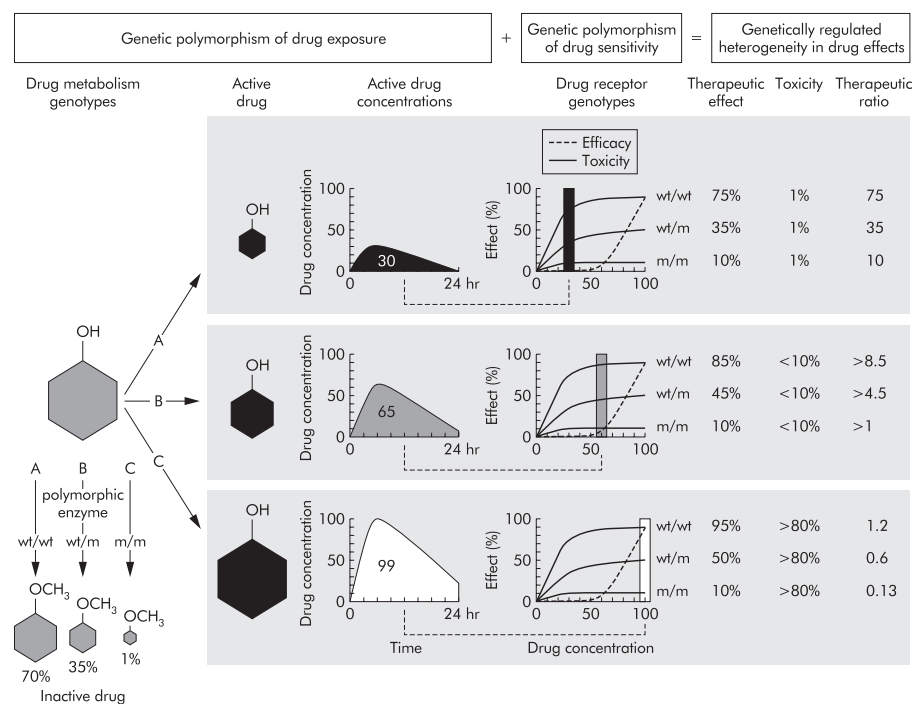
Drug metabolising enzymes
There are over 30 families of drug metabolising enzymes in humans,[1] and essentially all have genetic variants, many of which cause functional changes in the proteins encoded, and thereby change the metabolism of drugs.

Thiopurine methyltransferase (TPMT) and azathioprine, mercaptopurine, or thioguanine therapy
The genetic polymorphism of TPMT is one of the most well developed examples of clinical pharmacogenomics. TPMT catalyses the S-methylation of

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Correspondence to:
Dr W E Evans, St Jude
Children's Research
Hospital, 332 N
Lauderdale, Memphis, TN
38101–0318, USA;
william.evans@stjude.org
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Figure 1 Polygenetic determinants of drug response (reproduced with the publisher's permission, from Evans and Johnson, *Annu Rev Genomics Hum Genet* 2001;**2**:9–39).

the thiopurine agents azathioprine, mercaptopurine and thioguanine.[15 16] These agents are commonly used for a diverse range of medical indications, including leukaemia, rheumatic diseases, inflammatory bowel disease, and solid organ transplantation. The principal cytotoxic mechanism of these agents is generally considered to be mediated via the incorporation of thioguanine nucleotides (TGN) into DNA. Thus, thiopurines are inactive prodrugs that require metabolism to TGN to exert cytotoxicity. This activation is catalysed by a multi-enzyme pathway, the first of which is hypoxanthine phosphoribosyltransferase (HPRT). Alternatively, these agents can be inactivated via oxidation by xanthine oxidase or methylation by TPMT. In haematopoietic tissues, xanthine oxidase is negligible, leaving TPMT as the only inactivation pathway. TPMT activity is highly variable and polymorphic in all large populations studied to date; about 90% of individuals have high activity, 10% have immediate activity, and 0.3% have low or undetectable enzyme activity.[17 18] Numerous studies have shown that TPMT deficiency patients are at high risk for severe, and sometimes fatal, haematological toxicity.[6–8]

The molecular basis for polymorphic TPMT activity has now been defined for most patients. While eight TPMT alleles have been identified, three alleles (*TPMT*2, TPMT*3A, TPMT*3C*) account for about 95% of intermediate or low enzyme activity cases (fig 2).[16–20] All three alleles are associated with lower enzyme activity, because of increased rates of proteolysis of the mutant proteins.[21 22] The presence of *TPMT*2*, *TPMT*3A*, or *TPMT*3C* is predictive of phenotype; patients with one wild type allele and one of these variant alleles (that, heterozygous) have intermediate activity and patients inheriting two variant alleles are TPMT deficient.[17 18] While most studies have used erythrocytes as a surrogate tissue for measuring TPMT activity, studies have also shown that TPMT genotype determines TPMT activity in leukaemia cells, as would be expected for germline mutations.[23] By using allele specific PCR or PCR-RFLP to detect the three signature mutations in these alleles, a rapid and comparatively inexpensive assay is available to identify >90% of all mutant alleles.[17] In white populations, *TPMT*3A* is the most common mutant *TPMT* allele (3.2%–5.7% of *TPMT* alleles), while *TPMT*3C* has an allele frequency of 0.2%–0.8% and *TPMT*2* represents 0.2%–0.5% of *TPMT* alleles.[16 17] Studies in white, African, and

Asian populations have demonstrated the broad utility of this approach,[24–27] while revealing that the frequency of these mutant TPMT alleles differs among various ethnic populations. For example, East and West African populations have a frequency of mutant alleles similar to white populations, but all mutant alleles in the African populations are *TPMT*3C*.[25] Among African-Americans, *TPMT*3C* is the most prevalent allele, but *TPMT*2* and *TPMT*3A* are also found, reflecting the integration of white and African-American genes in the US population.[24] In Asian populations, *TPMT*3C* is the predominant mutant allele (100% of mutant alleles in published studies to date).[16 27]

Interest in TPMT pharmacogenetics has been fuelled by the finding that TPMT genotype identifies patients who are at risk of toxicity from mercaptopurine or azathioprine. Patients with a homozygous mutant or compound heterozygous genotype are at very high risk of developing severe haematopoietic toxicity, if treated with conventional doses of thiopurines.[8 28] More recent studies have now shown that patients who are heterozygous at the TPMT gene locus are at intermediate risk of dose limiting toxicity.[6 7] In a study of azathioprine for rheumatic disease, patients with wild type TPMT received treatment for a median 39 weeks without complications compared with a median of two weeks in patients heterozygous for one mutant TPMT allele and one wild type allele.[6] A second study in Japanese rheumatic disease patients receiving azathioprine recently confirmed the importance of a heterozygous TPMT genotype for predicting systemic toxicity.[29] A more quantitative analysis of mercaptopurine for childhood ALL found that TPMT deficient patients tolerated full doses of mercaptopurine for only 7% of weeks, whereas heterozygous and homozygous wild type patients tolerated full doses for 65% and 84% of scheduled weeks of treatment over the 2.5 years of treatment, respectively.[6] The percentage of weeks in which mercaptopurine dose had to be decreased to prevent toxicity was 2%, 16%, and 76% in wild type, heterozygous, and homozygous mutant individuals.[6] Collectively, the above studies show that the influence of TPMT genotype on haematopoietic toxicity is most dramatic for homozygous mutant patients, but is also of clinical relevance for heterozygous individuals, which represent about 10% of patients treated with these drugs.

Prospective determination of functional TPMT status is of clinical utility to prevent mercaptopurine and azathioprine

Table 1 Examples of genetic polymorphisms of human drug metabolising enzymes and transporters

Enzyme	Substrates	Consequences of polymorphism for drug effects	Reference
Phase I enzymes			
CYP2C9	tolbutamide, warfarin, phenytoin, non-steroidal anti-inflammatories	Anticoagulant effect of warfarin	(76–79)
CYP2C19	mephenytoin, omeprazole, hexobarbital, mephobarbital, propranolol, proguanil, phenytoin	peptic ulcer response to omeprazole	(80–81)
CYP2D6	β blockers, antidepressants, antipsychotics, codeine, debrisoquin, dextromethorphan, encainide, flecainide, fluoxetine, guanoxan, methoxy-amphetamine, N-propylajmaline, perhexiline, phenacetin, phenformin, propafenone, sparteine	tardive dyskinesia from antipsychotics, narcotic side effects, efficacy, and dependence, imipramine dose requirement, β blocker effect	(31,32,36,82–93)
CYP3A4/3A5/3A7	macrolides, cyclosporin, tacrolimus, calcium channel blockers, midazolam, terfenadine, lidocaine, dapsone, quinidine, triazolam, etoposide, teniposide, lovastatin, alfentanil, tamoxifen, steroids, benzo[a]pyrene	not yet elucidated, polymorphic 3A5 expression linked to 3A5 promoter polymorphism	(94–97)
Dihydropyrimidine dehydrogenase	Fluorouracil	5-fluorouracil neurotoxicity	(98–99)
Phase II enzymes			
N-acetyltransferase (NAT2)	isoniazid, hydralazine, sulfonamides, amonafide, procainamide, dapsone, caffeine	hypersensitivity to sulfonamides, amonafide toxicity, hydralazine induced lupus, isoniazid neurotoxicity	(100–105)
Glutathione transferases GSTM1, M3, T1	Several anticancer agents	Decreased response in breast cancer; more toxicity and worse response in AML	(48–50)
Thiopurine methyltransferase	Mercaptopurine, thioguanine, azathioprine	Thiopurine toxicity and efficacy, risk of second cancers	(8,17,18,28,106–115)
UDP-glucuronosyl-transferase UGT1A1	Irinotecan, bilirubin	Irinotecan glucuronidation	
Transporters			
MDR-1	Natural product anticancer drugs, HIV protease inhibitors, digoxin	Decrease CD4 response in HIV infected patients, decreased digoxin AUC	(59, 64)

toxicity. TPMT genotyping is now available as a molecular diagnostic from reference laboratories, representing the first CLIA certified pharmacogenomics test for individualising drug treatment based on a patient's genotype. Patients with a "low methylator" status (homozygous mutant or compound heterozygote) may tolerate standard doses, but are at significantly greater risk of toxicity, often necessitating a lower dose of these drugs (50%–80% of standard doses).[6 8]

Cytochrome P450 enzymes

The cytochrome P450 enzymes represent a large family of drug metabolising enzymes,[1] catalysing the metabolism of more medications than any other family of enzymes. Debrisoquin hydroxylase (*CYP2D6*) is probably the most well characterised genetic polymorphism in cytochrome P450 enzymes, representing the first human polymorphic drug metabolising enzyme to be cloned and characterised at the molecular level.[30] As was common in the pre-genomics era, its discovery was in part serendipitous, facilitated by the principal investigator's development of marked hypotension during participation in a pharmacokinetic study of debrisoquine, an antihypertensive.[31] Family studies subsequently showed that he had inherited a deficiency in debrisoquin metabolism, an enzyme deficiency discovered independently with sparteine.[32] Many drugs (>30) were subsequently found to be substrates for CYP2D6, and this genetic polymorphism was documented in most populations worldwide, with pronounced racial differences in mutant allele frequencies. A large number of *CYP2D6* SNPs, gene deletions and gene duplications have now been discovered, and concordance between genotype and phenotype has been well established for many drug substrates.[33] CYP2D6 deficiency can result in either exaggerated drug effects when CYP2D6 is the major inactivation pathway (for example, tricyclic antidepressants, fluoxetine) or diminished effects when CYP2D6 is required for activation (for example, codeine).[34 35] Moreover, gene duplication of CYP2D6 leads to inheritance of an "ultrarapid metaboliser" phenotype, which has been linked to treatment failure for some antidepressant and antipsychotic drugs.[36 37]

Genetic polymorphisms do not always translate into distinct phenotypic differences in drug metabolism in population studies, exemplified by a common polymorphism in the P450 enzyme CYP3A5.[38] The CYP3A5 protein is expressed in only about half of African-Americans and about 20% of the white population, and those people who express both CYP3A5 and CYP3A4 have higher total CYP3A enzyme activity, which translates to higher rates of drug clearance when medications are metabolised by both CYP3A4 and CYP3A5 as the major route of elimination. Recently, the genetic basis for polymorphic CYP3A5 expression was discovered; a SNP located over >1600 bp into intron 3 of CYP3A5 (and more than 200 nucleotides 5' of wild-type exon 4 splice site), leading to insertion of >130 nucleotides of intron 3 sequence into the mRNA. This additional mRNA sequence introduces an early stop codon that encodes a truncated non-functional CYP3A5 protein. For drugs that are equally metabolised by both, the net rate of metabolism is the sum of CYP3A4 and CYP3A5, partially masking the genetic polymorphism of CYP3A5 (fig 3). The CYP3A pathway of drug elimination is also influenced by SNPs in the CYP3A4 gene, which changes the activity of this enzyme for some substrates but not others.[39] Discovery of the genetic basis for CYP3A5 deficiency,[38] makes it possible to easily identify those patients who express CYP3A5 based on their genotype, but the clinical importance of these CYP3A genetic polymorphisms has not been fully elucidated to date.

Glutathione S-transferases

Glutathione is conjugated to many electrophiles, including several medications and their potentially damaging oxidative
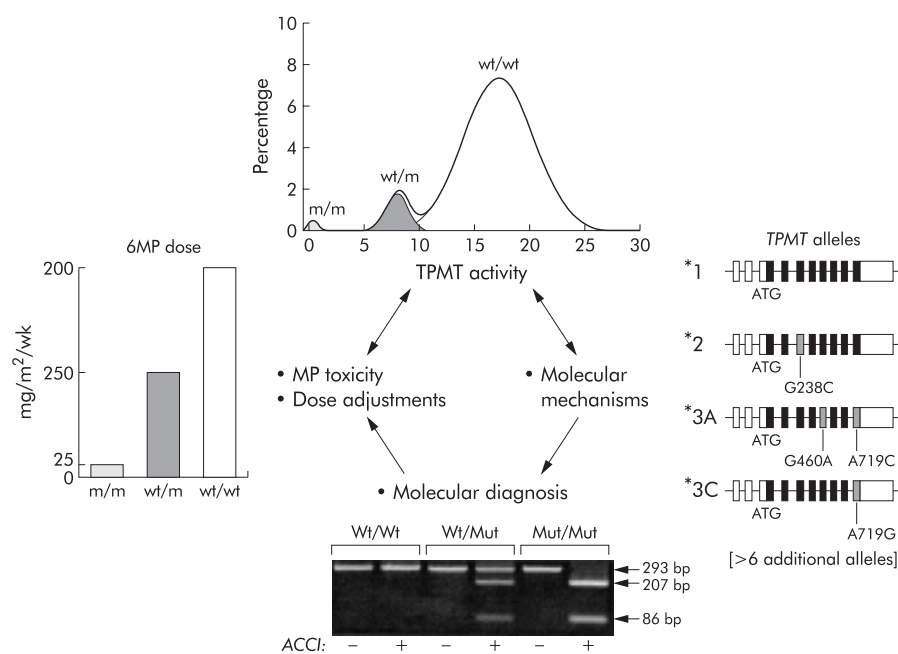
Figure 2 Genetic polymorphism of thiopurine methyltransferase and its importance in determining response to thiopurine medications (azathioprine, mercaptopurine, and thioguanine) (reproduced with publisher's permission, from Krynetski and Evans *Am J Hum Genet* 1998;**63**:11–16).

metabolites.[40] [41] Conjugation with glutathione generally inactivates these reactive moieties,[42] although this is not always the case. These conjugation reactions are catalysed by a family of human glutathione S-transferases (GST), and the human genes encoding these enzymes are highly polymorphic, with about 50% and 25% of most populations having a complete deletion of *GST-M1* and *GST-T1*, respectively, rendering them void of these enzyme activities. As is typical for many gene polymorphisms, there are important racial and ethnic differences in the frequencies of gene deletions in different human populations. Other GSTs, (that is, *GST-P1* and *GST-A*), are also subject to genetic polymorphisms, and these have been implicated in resistance to several anti-anticancer agents.[41] [43] [44]

Several studies have reported associations between GST polymorphisms and the efficacy and/or toxicity of cancer chemotherapy. High GST activity has been associated with resistance to anticancer agents, consistent with the association of inherited GST deficiencies with a decreased risk of haematological relapse[45] [47] and central nervous system relapse[46] [48] and to improved prednisone response[47] [49] in children treated with combination chemotherapy for acute lymphoblastic leukaemia. Similarly, inheriting a *GST-P1* allele coding for the Ile[105]Val amino acid substitution has been associated with improved overall breast cancer survival, compared with patients who had at least one wild type *GST-P1* allele.[48] [50] In breast cancer patients, deletion of either *GST-M1* or *GST-T1* was associated with improved survival, with further improvement in outcome if both genes were deleted.[49] [51] In contrast, in patients with acute myeloid leukaemia treated with high doses of combination chemotherapy, the homozygous *GST-T1* deletion was associated with a higher risk of toxic death during remission,[50] most probably because such patients could not tolerate intensive chemotherapy due to the absence of detoxifying GST enzymes. Together, these studies in breast cancer and AML patients illustrate that the importance of a genetic polymorphism in drug metabolism may differ based on the nature and intensity of the treatment regimen being prescribed. When treatment intensity is comparatively modest, leading to potential under treatment of some patients, then inheriting an enzyme deficiency can increase the exposure to drugs that are substrates, and thereby increase their efficacy. Conversely, when drugs are being dosed at levels that are near those that produce toxicity (common in AML),

then inheritance of an enzyme deficiency can lead to a worse outcome because of greater toxicity.

Drug transport proteins
Transport proteins play an important part in human physiology and pharmacology, providing protective functions (for example, blood-brain barrier), contributing to critical cellular processes (for example, biliary excretion), and influencing the absorption of drugs and other compounds from the small intestine. The ATP binding cassette (ABC) family of membrane transporters,[51] comprise an extensively studied group of transporters influencing drug disposition and effects. P-glycoprotein (PGP), a member of the ABC family, is involved in the energy dependent efflux of substrates, including bilirubin, several anticancer drugs, cardiac glycosides, immunosuppressive agents, glucocorticoids, HIV-1 protease inhibitors, and many other drugs.[51–55] Expression of the PGP gene (*ABCB1*, also names *MDR1*) in normal tissue suggests that it is involved in excreting drugs and their metabolites into urine, bile, and the intestinal lumen.[54–58] At the blood-brain barrier, PGP in the choroids plexus limits brain accumulation of many drugs, including cyclosporin A, dexamethasone, digoxin, domperidone, loperamide, and vinblastine (table 2).[55] [57]

PGP expression differs markedly among individuals,[58] [60] the molecular basis of which has not been fully elucidated. Recently, a synonymous SNP in exon 26 (3435C>T), was reported to be associated with duodenal PGP protein expression; patients homozygous for the T allele had more than twofold lower duodenal PGP expression compared with patients with CC genotypes.[58] [60] Clinical pharmacokinetic studies of digoxin, a PGP substrate, demonstrated significantly higher bioavailability in patients with the CC genotype.[59] As is common for most genetic traits, there are considerable ethnic differences in the frequency of the 3435C>T SNP; the TT genotype was found in 0%–6% of black African and African-Americans, 20–47% of Asians, and 24%–36% of the white population.[60–62] However, the 3435C>T SNP is in linkage disequilibrium with a non-synonymous SNP in exon 21 (1236C>T, Ala893Ser) that has been shown to change PGP function,[63] so it is unclear whether the 3435C>T SNP is of functional importance or just in linkage with the functionally important SNP in *ABCB1*.
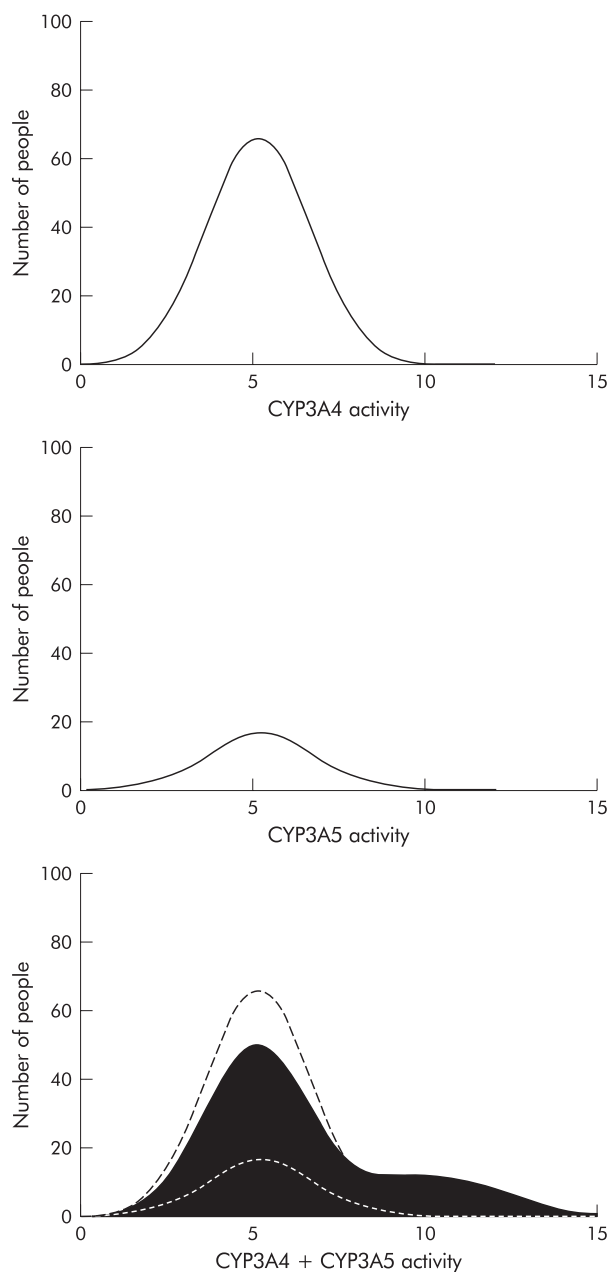
Figure 3 The cytochromes P450 CYP3A4 and CYP3A5 genetic polymorphism. The top panel depicts the distribution of CYP3A4 activity in the white population, assuming that 100% of individuals express CYP3A4, with a 10-fold range of activity in the population. The middle panel depicts CYP3A5 expression, assuming that 25% of the white population express 3A5, with a 10-fold range of activity. The bottom panel depicts the CYP3A4 and CYP3A5 distributions (dashed lines) and the composite distribution for drugs metabolised equally well by both enzymes.

In a recent study, the *ABCB1* 3435C>T polymorphism was found to be associated with significant differences in nelfinavir and efavirenz pharmacokinetics in HIV infected patients, and recovery of CD4 count was significantly greater and more rapid in patients with the TT genotype than patients with either CT or CC genotypes. Of all variables evaluated, only *ABCB1* genotype and baseline HIV RNA copy number were significant predictors of CD4 recovery.[64] Unfortunately, these investigators did not genotype for the *ABCB1* 1236C>T SNP, so it remains unclear whether the 3435C>T is causative or in linkage with the causative SNP. This is the first evidence that a host genetic marker can predict immune recovery after ini-

tiation of antiretroviral treatment, suggesting a potential strategy to individualise HIV therapy, if these findings can be independently verified.

GENETIC POLYMORPHISM OF DRUG RECEPTORS AND OTHER TARGETS

Genetic variation in drug targets (for example, receptors) can have a profound effect on drug effects, with over 25 examples already identified, including the β_2 adrenoceptor and response to β_2 agonists,[9 65 66 68] angiotensin converting enzyme and renoprotective effects of ACE inhibitors,[66] apolipoprotein E and response to HMG-Co reductase inhibitors ("statins")[67] and more than 20 other examples (reviewed in references[1–3]). The potential importance of these genetic polymorphisms is exemplified in this review by the β_2 adrenoceptor.

Genetic polymorphism of the β_2 adrenoceptor exemplifies a well characterised and clinically relevant polymorphism in a drug target.[65] The β_2 adrenoreceptor is a G protein coupled receptor that interacts with various medications and endogenous catecholamines. These receptors are widely expressed in humans and play an important part in regulating cardiac, vascular, pulmonary, and metabolic functions.[9 65] Studies of such physiological functions of the human β_2 adrenoceptor have revealed substantial interpatient differences in receptor function and responsiveness to stimulation. In the heart, activation of β_2 adrenoceptor results in an increased rate and force of cardiac muscle, whereas β_2 adrenoceptor stimulation in the lungs acts to relax airway smooth muscle. Effects on lipolysis in subcutaneous fat have also been reported, mediated putatively through regulation of lipid mobilisation, energy expenditure, and glycogenolysis. Insights to the molecular basis for inherited differences in the β_2 adrenoceptor have been illuminated by the discovery of several SNPs in the *B2AR* gene, and their association with altered expression, down regulation, or coupling of the receptor.[65] 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 comparatively common (allele frequency = 0.4–0.65) and are being extensively investigated for their clinical importance. Studies of agonist mediated vasodilatation and desensitisation have begun to dissect the relative contribution of the codon 16 and codon 27 mutations.[9] Subjects who were homozygous for Arg16 had nearly complete desensitisation after continuous infusion of isoproterenol, with venodilatation decreasing from 44% at baseline to 8% at 90 minutes. Homozygous Gly16 patients had no significant change in venodilatation, regardless of their codon 27 sequence. Polymorphism at codon 27 was also of functional relevance, as patients homozygous for Glu27 had higher maximal venodilatation in response to isoproterenol (86%) than observed in subjects with the codon 27 Gln genotype, regardless of codon 16 sequence.[9]

These results are consistent with the reported effects of B2AR genotype on pulmonary response to acute or chronic β agonist therapy. The FEV_1 response to a single dose of oral albuterol was more that sixfold higher in patients with an Arg/Arg genotype at codon 16 compared with Gly/Gly patients, even though similar plasma drug concentrations were achieved.[68] Interestingly, the influence of this genotype changed when long term inhaled β agonist therapy was used. An Arg/Arg genotype in patients on regularly scheduled β agonist therapy resulted in a gradual decline in morning peak expiratory flow (AM PEF) over the 16 weeks of evaluation, while no change in this parameter was observed in patients with a Gly/Gly genotype.[69] In addition, AM PEF deteriorated substantially after cessation of treatment in the Arg/Arg patients receiving regularly scheduled inhaled β agonist therapy, but not in patients with a Gly/Gly genotype.[69] There was no evidence that the codon 27 polymorphism influenced AM PEF in these patients. These data suggest that patients with a B2AR Arg/Arg16 genotype may be at risk for

Table 2 Examples of drug target pharmacogenetics

Gene/gene product	Drug	Drug effect associated with polymorphism	Reference
ACE	ACE inhibitors (eg, enalapril)	Renoprotective effects, BP reduction, left ventricular mass reduction, endothelial function improvement, ACE inhibitor induced cough.	(66, 117–125)
	Fluvastatin	Lipid changes (eg, reductions in total LDL-cholesterol and apolipoprotein B); progression/regression of atherosclerotic lesions.	
β_2 adrenergic receptor	β_2 agonists (eg, albuterol, terbutaline)	Bronchodilatation, susceptibility to agonist induced desensitisation, cardiovascular effects (eg, increased heart rate, cardiac index, peripheral vasodilatation).	(9, 68–70, 126–130)
Gs protein α	β blockers (eg, metoprolol)	Antihypertensive effect	(131)
Platelet FC receptor (FCRII)	Heparin	Heparin induced thrombocytopenia.	(132)
ALOX5	Leukotriene biosynthesis inhibitors (eg, ABT-761-zileuton derivative)	Improvement in FEV_1	(10)
Oestrogen receptor	Conjugated oestrogens	Bone mineral density increases.	(133)
Sulfonylurea receptor	Sulfonylureas (eg, tolbutamide)	Sulfonylurea induced insulin release.	(134)
Dopamine receptors (D2, D3, D4)	Antipsychotics (eg, haloperidol, clozapine, thioridazine nemorapride)	Antipsychotic response (D2, D3, D4), antipsychotic induced tardive dyskinsia (D3), antipsychotic induced acute akathisia (D3), hyperprolactinaemia in women (D2)	(73,135–141)
5HT2A, 5HT6	Antipsychotics (eg, clozapine, typical antipsychotics)	Clozapine response (5HT2A, 5HT6), typical antipsychotic response and long term outcomes (5HT2A).	(142–144)
G protein β3	Antidepressants (various)	Response to antidepressant therapy.	(145)
Serotonin transporter (5-HTT)	Antidepressants (eg, clomipramine, fluoxetine, paroxetine, fluvoxamine)	Clozapine effects, 5-HT neurotransmission, antidepressant response.	(73, 146–148)
Ryanodine receptor	Anaesthetics (eg, halothane)	Malignant hyperthermia	(149)

deleterious or non-beneficial effects of regularly scheduled inhaled β agonist therapy and may be candidates for alternative treatment or dosing schedules and/or earlier initiation of anti-inflammatory drugs. These results are consistent with the data described above for desensitisation of the β2 adrenoceptor in patients with a codon 16 Arg/Arg genotype.[9] Although the codon 27 polymorphism does not seem to have a significant effect on inhaled β agonist therapy, an association between the codon 16 Gln/Gln genotype and an increased incidence of obesity has been observed. This relation seemed to be more prominent in men and could be overcome with exercise.

These B2AR SNPs are not the only SNPs found in the β_2 adrenoceptor, with at least 13 distinct SNPs identified to date, leading to evaluation of B2AR haplotype structure compared with individual SNPs in determining receptor function and pharmacological response.[70] While over 8000 B2AR haplotypes are theoretically possible, only 12 distinct haplotypes were observed among 77 subjects of various ethnic origin.[70] Importantly, clinical evaluation of β agonist therapy in asthma patients revealed a better association of B2AR haplotype and bronchodilator response, than observed with any B2AR SNP alone.[70] This is not surprising, as haplotype structure is often a better predictor of phenotypic consequences, which has led to the development of various methods to determine haplotype structure, some of which are comparatively simple to perform.[71] It is likely that as more pharmacogenomic studies are conducted, haplotype structure will commonly emerge as the most informative genetic determinant of drug response.

MOLECULAR DIAGNOSTICS FOR OPTIMISING DRUG THERAPY

There is clearly great potential for pharmacogenomics to yield important new molecular diagnostics that will become routine clinical laboratory tests, by which physicians and pharmacists select drugs and doses for individual patients. Instead of using empirical treatment algorithms, pharmacogenomics can provide patient specific diagnostics to optimise drug treatment. Using the amount of DNA that can be isolated from a few millilitres of blood, it is possible to determine thousands of genotypes, and methodology is improving so rapidly that it will soon be straightforward to perform these tests in high throughput, automated systems, screening for thousands of SNPs in one test. This will be necessary, because most drug effects are polygenic in nature[1 72] so treatment decisions will be based on a panel of SNPs. These genotyping results will not be reported as a list of SNPs, rather they will be interpreted for the clinician according to the patient's diagnosis and treatment options. These new tests will not replace the more conventional biochemical tests that are currently used to assess organ function and disease status, rather they will provide additional tools for individualising and optimising drug therapy. Obviously, genotyping will not eliminate the need for follow up assessment of response, compliance with treatment, and other variables that influence treatment outcome. However, pharmacogenomics will make it increasingly possible to select the medications and doses that are optimal for each person, thereby improving efficacy and reducing toxicity.[1 73 74] In this regard, it has been recently reported[73] that among 27 drugs for which adverse events are frequently reported, a significantly higher percentage are metabolised by a polymorphic enzyme (59%) compared with randomly selected drugs (7%–22%). A substantial amount of work remains to be done, before the full clinical utility of pharmacogenomics can be fully appreciated and realised. While there are currently numerous examples that illustrate the potential, we are in the early days of deciphering the importance of genetic polymorphisms in determining drug response. Critical issues going forward include the fact that most drug effects are polygenic in nature, that haplotype structure is often more

informative than individual SNPs, and that functionally important polymorphisms can reside outside the coding or regulatory regions of genes. It is also important to recognise that clearly defined and objectively measured end points (that is, phenotypes) are essential to elucidate genotype-phenotype relations. In this regard, prospective clinical trials represent the ideal context within which pharmacogenomics of drug response are most likely to be elucidated. If done properly, ongoing research will change the practice of medicine and pharmacy, such that using genotype to individualise drug therapy will become the norm.

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