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COMMENTARIES

Drug-metabolizing enzymes: Evidence for clinical utility of pharmacogenomic tests

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Drug research and development have recently been hampered by high costs,[1] notably high investigational new drug (IND) failure rates[2] and multiple new drug application (NDA) review cycles.[3] The number of applications for new molecular entities submitted to the Food and Drug Administration (FDA) has declined steadily.[4] As part of the FDA's strategic plan,[5] the FDA is developing standards to apply emerging technologies (eg, pharmacogenomics) to provide effective translation of new scientific discoveries into safe and effective medical products. A recent document by the FDA stressed the following[4]: "The product development problems we are seeing today can be addressed, in part, through an aggressive, collaborative effort to create a new generation of performance standards and predictive tools. The new tools will match and move forward new scientific innovations and will build on knowledge delivered by recent advances in science, such as bioinformatics, genomics, imaging technologies, and materials science." There are various initiatives within the Center for Drug Evaluation and Research to address issues in the area of pharmacogenomics. A guidance for industry on genomic data submission has been published.[6,7] The guidance was intended to encourage voluntary genomic data submission by sponsors using

pharmacogenomics in exploratory research during drug development and to clarify under what circumstances genomic data submission is required. For example, the guidance discusses when to submit genomic biomarker data, on the basis of how the biomarker is used during the IND/NDA phase and the status of the biomarker (whether it is a "known valid," "probable valid," or "exploratory" biomarker). A workshop was held in November 2003 to discuss issues related to genomic data submissions, and the proceedings have been published.[8-11] In addition to the guidance on genomic data submissions, the FDA is developing a new guidance for drug-test combinations when a deoxyribonucleic acid–based test is used before a drug is prescribed. Another public workshop was held in July 2004 to identify issues in the development of these combination products,[12] and a concept paper was published.[13]

With the increasing knowledge and available tools in pharmacogenomics, the FDA will continue to encourage genomics-based research and the translation of the resultant scientific data to clinical practice.[14-16] On the basis of the FDA guidance,[6,7] data generated related to genomic biomarkers will need to be submitted for review in the NDA, with various reporting formats (full report, abbreviated report, or synopsis) that depend on the purpose of the genomic evaluation and the validity of the genomic biomarker.[6,7] The type of genomic data (eg, which alleles, what genotypes) that need to be evaluated is one of the critical issues in drug development and regulatory review[17] and is the subject of this commentary. Consideration of racial or ethnic differences in the distribution of various alleles with no or reduced metabolic activity in the evaluation of dose-response relationships is also discussed.

REVIEW OF CLINICAL PHARMACOLOGY AND LABELING

To optimize drug therapy and reduce adverse events, it is critical that information on how various intrinsic factors (age, gender, race, genetics, and others) and extrinsic factors (concomitant medication and others)[18,18a] may affect drug treatment be available for health care providers and patients. When a drug is being developed, variability in drug response and the factors contributing to it should be investigated, and this information should be included in the labeling. Detailed data are included in the "important clinical pharmacology findings" section, and key results are summarized in the "executive summary" section of the clinical pharmacology review.[19] For example, changes in pharmacokinetic parameters reflecting systemic exposure, such as area under the plasma concentration–time curve (AUC) or maximum plasma concentration, as a result of various extrinsic and intrinsic factors may be summarized and displayed in graphic or table forms. The clinical significance of altered systemic exposure resulting from these factors, including genetics, depends on the concentration-response relationships for both efficacy and toxicity.[20] If the concentration-response relationship is well described, knowledge of the effects of genotype, an intrinsic factor, can lead to rational adjustment of dose or dosing interval or to appropriate warnings and precautions. For example, the labeling of atomoxetine (Strattera; Eli Lilly & Co, Indianapolis, Ind), thioridazine (Mellaril; Novartis Pharmaceutical Corp, East Hanover, NJ), voriconazole (Vfend; Pfizer, New York, NY), 6-mercaptopurine (Purinethol; Gate Pharmaceutical, Sellerville, Pa), and irinotecan (Camptosar; Pharmacia & Upjohn, Kalamazoo, Mich) contains information about the genetics of metabolizing enzymes (eg, cytochrome P450 [CYP] enzymes CYP2D6 and CYP2C19, thiopurine *S*-methyltransferase, and uridine diphosphate–glucuronosyltransferase [UGT] 1A1) that are responsible for the elimination of these drugs to warn about genetic variation in drug disposition (Table I).[21]

APPLICATIONS OF PHARMACOGENETICS AND PHARMACOGENOMICS IN DRUG DEVELOPMENT AND REGULATORY REVIEW

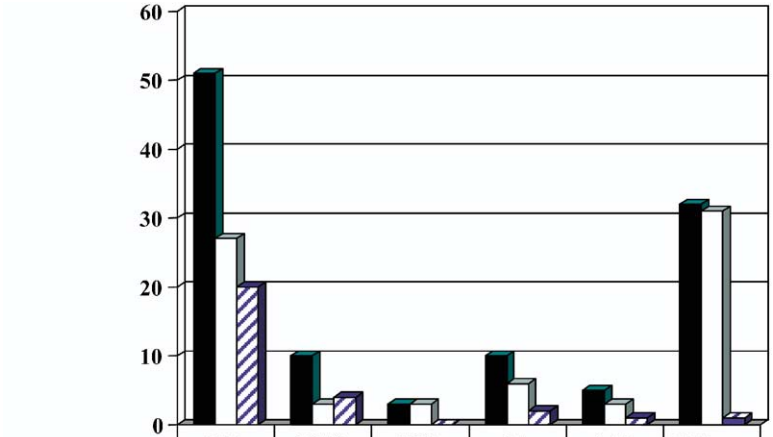
A recent internal, informal survey of the IND and NDA submissions received at the Center for Drug Evaluation and Research indicated that, of the 70 submissions with pharmacogenomic data received between 1992 and 2001, many evaluated the status of drug-metabolizing enzymes, with CYP2D6 being the most frequent. Fig 1 depicts the distribution of submissions evaluating various polymorphic enzymes.[22] Many of the submissions received between 1992 and 1999 used phenotyping (eg, urinary metabolic ratios of dextromethorphan and dextrorphan) to estimate CYP2D6 activity. Most of the later submissions (received between 2000 and 2001) used genotyping.

A number of enzymes listed in Fig 1, including CYP2D6, CYP2C9, CYP2C19, and UGT1A1, are "known valid" metabolizing enzyme biomarkers. A *known valid biomarker* is defined as being measured in an analytic test system with well-established performance characteristics and for which there is widespread agreement in the medical or scientific community about the physiologic, toxicologic, pharmacologic, or clinical significance of the results.[6,7] Fig 1 also includes en-

Table I. Examples of pharmacogenomic information regarding drug-metabolizing enzymes in drug label[21,215,216]

Brand name and generic name	*Labeling section*	*Labeling statement*
Purinethol (6-mercaptopurine) (July 2004 labeling)	Warnings	Individuals who are homozygous for an inherited defect in the thiopurine *S*-methyltransferase gene may be unusually sensitive to the myelosuppressive effects of mercaptopurine and prone to the development of rapid bone marrow suppression after the initiation of treatment. . . (see Dosage and administration section).
	Dosage and administration	Patients with little or no inherited thiopurine *S*-methyltransferase activity are at increased risk for severe Purinethol toxicity from conventional doses of mercaptopurine and generally require substantial dose reduction. The optimal starting dose for homozygous deficient patients has not been established (see Clinical pharmacology and Warnings and precautions sections).
Vfend (voriconazole) (April 2004 labeling)	Clinical pharmacology	In vivo studies indicated that CYP2C19 is significantly involved in the metabolism of voriconazole. This enzyme exhibits genetic polymorphism. For example, 15% to 20% of Asian populations may be expected to be PMs. For white subjects and black subjects, the prevalence of PMs is 3% to 5%. Studies conducted in white and Japanese healthy subjects have shown that PMs have, on average, 4-fold higher voriconazole exposure (AUC_τ) than their homozygous EM counterparts. Subjects who are heterozygous EMs have, on average, 2-fold higher voriconazole exposure compared with their homozygous EM counterparts.
Mellaril (thioridazine) (July 2003 labeling)	Contraindications	Thioridazine is contraindicated . . . in patients, comprising about 7% of the normal population, who are known to have a genetic defect leading to reduced levels of activity of CYP2D6 (see Warnings and precautions section).
Strattera (atomoxetine) (March 2003 labeling)	Drug-drug interactions	In EMs inhibitors of CYP2D6 increase atomoxetine steady-state plasma concentrations to exposures similar to those observed in PMs. Dosage adjustment of Strattera in EMs may be necessary when coadministered with CYP2D6 inhibitors (eg, paroxetine, fluoxetine, and quinidine) (see Drug interactions section under "Precautions"). In vitro studies suggest that coadministration of CYP inhibitors to PMs will not increase the plasma concentrations of atomoxetine.
	Laboratory tests	With regard to CYP2D6 metabolism, PMs of CYP2D6 have a 10-fold higher AUC and a 5-fold higher peak concentration to a given dose of Strattera compared with EMs. Approximately 7% of the white population are PMs. Laboratory tests are available to identify CYP2D6 PMs. The blood levels in PMs are similar to those attained by taking strong inhibitors of CYP2D6. The higher blood levels in PMs lead to a higher rate of some adverse effects of Strattera (see Adverse reactions section).
Camptosar (irinotecan)	Clinical pharmacology, warning, and dosage and administration	Patients who were homozygous for *UGT1A1*28* had a higher exposure to SN-38 than patients with the wild-type UGT1A1 allele. Individuals homozygous for the *UGT1A1*28* allele are at increased risk for neutropenia after Camptosar administration. A reduction in the starting dose by 1 level may be considered in patients aged >65 y, prior radiotherapy, performance status 2, increased bilirubin levels. A reduction in the starting dose by at least 1 level of Camptosar should be considered for patients known to be homozygous for the *UGT1A1*28* allele. . . . The appropriate dose reduction in this patient population is not known.

For additional information on thiopurine *S*-methyltransferase, see references 215 and 216.
EM, Extensive metabolizer; PM, poor metabolizer; AUC, area under plasma concentration–time curve; UGT, uridine diphosphate–glucuronosyltransferase.



	2D6	2C19	2C9	3A	1A2	Others
Total	51	10	3	10	5	32
Genotyping	27	3	3	6	3	31
Phenotyping	20	4	0	2	1	1

Fig 1. Distribution of pharmacogenetic-pharmacogenomic studies evaluating the impact of different genotypes and phenotypes of CYP2D6, CYP2C19, CYP2C9, CYP3A, and CYP1A2 and other metabolizing enzymes, transporters, or receptors (including *ABCB1* [multidrug resistance 1 (*MDR1*)] gene product P-glycoprotein, uridine diphosphate–glucuronosyltransferase 1A1, and other transferases and proteins) on the new drug's pharmacokinetics, pharmacodynamics, or efficacy-safety measures for 70 investigational new drugs (INDs) and new drug applications (NDAs) submitted between 1992 and 2001.[22]

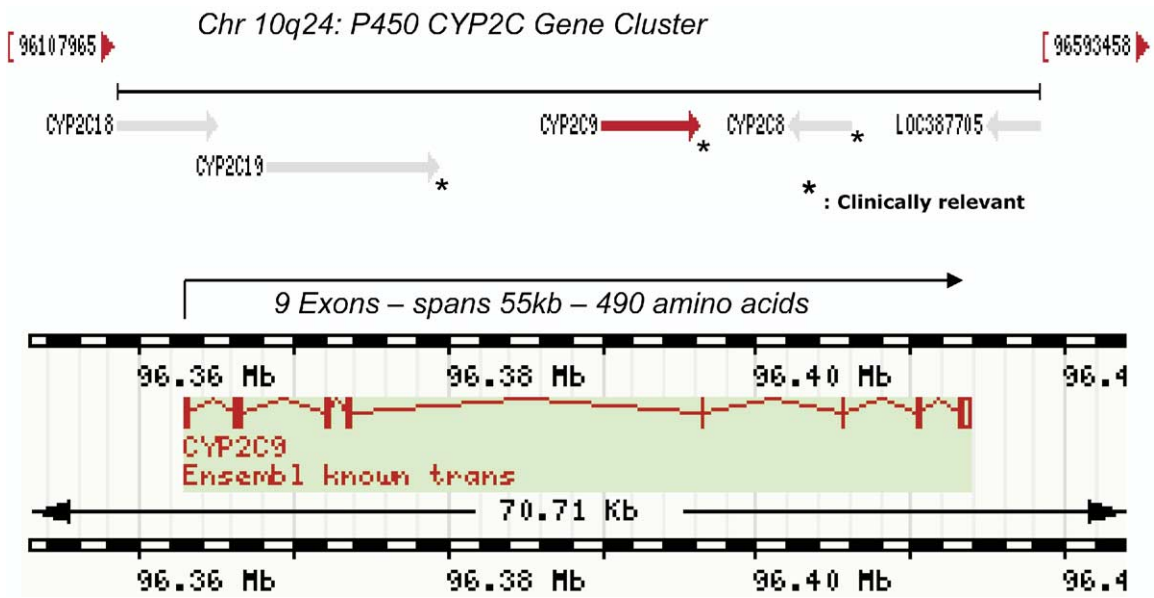


Fig 2. Genomic structure for *CYP2C9*.

zymes, transporters, and receptors or proteins that have not reached the "valid" biomarker status and are considered "exploratory" biomarkers. For example, for some genes (eg, *CYP3A4*), the correlation between certain genotypes and enzyme or transporter activities has been observed in vitro only.[23] For others (eg,

ABCB1), contradictory data have been published for different drugs and the correlation between single-nucleotide polymorphism (SNP) genotype or haplotype and the phenotype (pharmacokinetic parameters, other response measures) will need to be further defined. For many of these genes, the relationship between in vitro and in vivo phenotype data, in vivo genotype-phenotype correlations, the ethnic distribution of major alleles, and recommendations for specific alleles that might be genotyped in regulatory studies are discussed later.

CYP2C9

The *CYP2C9* gene is located at chromosomal position 10q24 in a multigene cluster consisting of other CYP2C subfamily members including *CYP2C18*, *CYP2C19*, and *CYP2C8* assembled as shown in Fig 2.[24] The *CYP2C9* gene spans some 55 kilobases and consists of 9 exons that encode a 490–amino acid protein. The clinically relevant genes, *CYP2C19*, *CYP2C9*, and *CYP2C8,* are highly homologous at the nucleotide level. These genes also exhibit genetic polymorphisms, which confer important clinical differences in the metabolism of known CYP2C substrates. The high degree of homology can introduce complexities in gene-based assays, yet critical primer design enables comprehensive evaluation of the genetic differences present in individuals.

The CYP2C9 protein represents the primary CYP2C protein present in the human liver and accounts for approximately 20% of the hepatic CYP content.[25] CYP2C9 plays a major role in the metabolism of numerous therapeutics including the antidiabetics glipizide and tolbutamide, the anticonvulsant phenytoin, the angiotensin II receptor antagonist losartan, the 3-hydroxy-3-methylglutaryl–coenzyme A reductase inhibitor fluvastatin, many nonsteroidal anti-inflammatory agents, and the commonly administered anticoagulant warfarin.

In vitro and in vivo correlations. Individual variation within the *CYP2C9* gene locus has been characterized because of the importance of this enzyme in the metabolism of common medicines. The variant forms are defined in Table II. The most common variants, *CYP2C9*2* and **3*, represent the predominant alleles with clinical consequences. In contrast to the *CYP2D6* poor metabolizer (PM) alleles, which result in nonfunctional alleles, the proteins encoded by the *CYP2C9* allelic variants exhibit differing affinity (Michaelis-Menten constant) or intrinsic clearance (maximum velocity/Michaelis-Menten constant) for differing substrates. This is exemplified by the *CYP2C9*2* allele, which results in impaired 6-/7-hydroxylation of *S*-warfarin; small effects, if any, on maximum velocity for tolbutamide; and no effect on the methyl hydroxylation of torsemide (INN, torasemide). Whereas *CYP2C9*2* effects appear to be more substrate-specific, the *CYP2C9*3* variants demonstrate reduced catalytic activity across the majority of CYP2C9 substrates, with lowered maximum catalytic rates or lower affinity for substrates in general.

Table II. Nomenclature for *CYP2C9* alleles[26-30]

CYP2C9 allele	*Effect of nucleotide change*	*Reference*
**1*	Wild type	
**2*	Arg144Cys	26
**3*	Ile359Leu	27
**4*	Ile359Thr	28
**5*	Asp360Glu	29
**6*	Del Aden818	30

Clinical relevance. An important clinical consequence of *CYP2C9* polymorphic variation is demonstrated by the individual differences in the metabolism of warfarin, a common oral anticoagulant. Individuals receiving warfarin therapy often demonstrate difficulties in initial dosing predictions, as well as maintenance dosing regimens. The in vivo consequences of the *CYP2C9* genotype and dosing requirements were documented by Aithal et al[31] in a study examining patients from an anticoagulation clinic requiring low maintenance doses of warfarin (<1.5 mg/d). In this study individuals who required a low dose of warfarin to maintain anticoagulation were about 6-fold more likely to possess a variant allele of the *CYP2C9* gene compared with unselected patients receiving the same therapy and a control population. Furthermore, those individuals in the low-dose group had significant difficulty in achieving optimal warfarin exposure and an increased risk of bleeding events. Subsequent studies have confirmed the important relationship between *CYP2C9* genotype and warfarin dosing, anticoagulation effects, and bleeding events.[32,33]

What alleles to measure. The *CYP2C9* allele frequencies have been well characterized in the major ethnic groups, with the 2 most common polymorphisms being identified as the **2* and **3* alleles. The *CYP2C9*2* allele occurs at an allelic frequency of approximately 10% in white subjects and 2% to 4% in black subjects and has not been seen in the Asian populations examined. The *CYP2C9*3* allele occurs at an allele frequency of 8% in white subjects, less than 1% in black subjects, and approximately 2% in Asians.

Table III. Summary of recommended polymorphic alleles of specific metabolizing biomarkers to measure in specific population groups[24,35-37]

Enzyme	*Basic alleles to measure in all population groups*	*Additional alleles relevant to specific population groups*		
		White†	*Black†*	*Asian Americans†*
CYP2C9	**2*, **3*		**5*, **6*	
CYP2C19	**2*, **3*	**4*, **5*, **6*		
CYP2D6	**3*, **4*, **5*, **6*, **2xN*	**10* (**41*)	**17* (**60*)	*10 (**21*) (**6*)
UGT1A1	**28*			

†Additional alleles to measure in this specific population group are shown, with possible additional alleles to measure in parentheses.

Other identified *CYP2C9* alleles occur at significantly reduced frequencies, including the Ile359Thr **4* allele identified in Japanese subjects and the Asp360Glu **5* and Del **6* alleles identified in black subjects.

Whereas research studies on metabolic differences imparted by genetic variation in CYP family members have often focused on coding regions of these genes, the contributions of genetic variation within regulatory regions are beginning to be appreciated. Recently, Shintani et al[34] characterized the impact of 7 SNPs located in the upstream regulatory region of the *CYP2C9* gene. Combinations of these SNPs have been characterized in promoter/reporter constructs transfected into cells and demonstrate reduced gene transcription of the reporter gene, suggesting that *CYP2C9* promoter variation may also play a role in reduced metabolism of substrates. Further studies are required to define the in vivo consequences of these common variants.

Conclusions. In considering substrates of CYP2C9, the common alleles *CYP2C9*2* and **3* account for the majority of intersubject variation. These common alleles could be routinely examined for defining the in vivo relationships between substrate metabolism and *CYP2C9* genotype. Table III lists the recommended polymorphic alleles to measure in specific population groups for CYP2C9, along with CYP2C19, CYP2D6, and UGT1A1 (which will be discussed in detail later).[24,35-37]

CYP2C19

PMs of *S*-mephenytoin do not express CYP2C19 because of a defective or mutated gene.[35,38-41] More than 10 mutated alleles for CYP2C19 are known, of which **2* and **3* are the most common.[42] Per definition, in a PM both alleles are mutated, whereas individuals with 1 mutated and 1 wild-type allele (heterozygotes) or 2 wild-type alleles (homozygotes) are extensive metabolizers (EMs). The PM frequency varies, with more PMs among Asians (approximately 15%) than among white subjects and black subjects (approximately 3%).[43] Extreme values have been reported in smaller ethnic groups such as Cuna Indians in Panama (0%)[44] and Vanuatuans in the South Pacific (approximately 70%).[45] There have been indications in the literature that a subgroup of super-rapid metabolizers exists, suggesting that those may be found among nonresponders to treatment with the CYP2C19 substrate proton pump inhibitors (PPIs). This phenomenon may be caused by a variant of the wild-type allele.[46] A wide array of drugs are known substrates of CYP2C19.[43]

In vitro and in vivo correlations. Several experimental settings have been used to demonstrate that in vitro data can be used to predict PM status in vivo. When different mutated alleles were compared with the wild-type allele in a bacterial expression system, good correlations were found with carbon monoxide binding spectra or Western blotting or by simply measuring the *S*-mephenytoin hydroxylase activity in recombinant enzymes.[47-50]

In vivo genotype-phenotype correlations. There are many good examples in vivo in which correlations between genotype and phenotype have been demonstrated. A good correlation with a clear cutoff level for EMs and PMs was found between the *S/R*-mephenytoin urinary ratio or the mephenytoin hydroxylation index and *CYP2C19* allele frequency in both Asians (Filipinos) and Saudis.[51] The urinary excretion of cycloguanil pamoate (INN, cycloguanil embonate), the primary chloroguanide (INN, proguanil) metabolite, as well as the ratio between chloroguanide and cycloguanil pamoate in urine, also correlated with EM and PM status determined by *CYP2C19* genotyping.[52] There is a clear correlation between mephenytoin *S/R* ratio and omeprazole metabolizer status,[53] consistent with the correlation between *CYP2C19* genotype and omeprazole metabolizer status (ratio between AUC or 3-hour concentrations of racemate omeprazole and its hydroxy

metabolite).[54,55] Like racemate omeprazole, other PPIs such as lansoprazole and pantoprazole are metabolized by CYP2C19 to a similar degree[56] and could, therefore, be used for phenotyping.[57] However, for mephenytoin, chloroguanide, and PPIs, there is an overlap between homozygous and heterozygous EMs. Because the *S*-omeprazole enantiomer is less dependent on CYP2C19, it is not as useful for phenotyping.

Which alleles to determine. By genotyping for **2* and **3* alleles, one would detect 84% of PMs among white subjects, greater than 90% among black subjects, and approximately 100% among Asians.[42,49,51] By also including **4* to**6* alleles, 92% of white PMs would be detected. The number of alleles to be included in genotyping should be based on a cost/benefit analysis. In contrast to CYP2C9, all compounds identified as CYP2C19 substrates to date are metabolized equally poorly in all PMs, irrespective of variant alleles or ethnic origin.[58] The only factor that seems to determine the difference in exposure between EMs and PMs for CYP2C19 substrates is the proportion of the drug metabolized by CYP2C19.[56]

Clinical relevance. The clinical relevance of polymorphic expression of CYP2C19 has to be evaluated separately for each drug, mainly on the basis of the proportion of dose that is metabolized via CYP2C19 in combination with the therapeutic index of the drug, as well as the consequences of suboptimal treatment.[59-83]

Tricyclic antidepressants (TCAs) (eg, amitriptyline) are partly metabolized by CYP2C19 and show higher plasma concentrations in PMs than in EMs.[43,60-66] No direct correlation between metabolizer status and adverse effect has been demonstrated, but there is an obvious risk because there is a correlation between plasma levels and toxic effect,[67,68] especially if CYP2D6, the major TCA pathway, is compromised.[69-71] Selective serotonin reuptake inhibitors (eg, citalopram) are also partly metabolized by CYP2C19, and accordingly, higher plasma concentrations have been reported in PMs than in EMs.[72-74]

CYP2C9 is the major metabolizing enzyme for phenytoin and warfarin, both with a narrow therapeutic window, but they are also partly metabolized by CYP2C19.[76,77] Patients who are both CYP2C19 PMs and CYP2C9 PMs are at risk of adverse effects. Because diazepam has a wide therapeutic window, there is no concern with the 2-fold higher exposure in PMs compared with EMs.[78,79] Also, because the degree of decrease in diazepam clearance with CYP2C19 inhibition correlates with the baseline clearance, patients with the highest exposure initially will have the least increase with CYP2C19 inhibition.[80]

For PPIs, the clinical relevance is dependent on dose; a clear gene-dose effect in clinical efficacy was demonstrated for 20 mg omeprazole, 10 mg rabeprazole, or 30 mg lansoprazole[81-83] but not for 40 mg omeprazole, which provides exposure high up on the dose-response curve (AstraZeneca, Mölndal, Sweden; data on file).

Conclusions. More than 10 different variant alleles of CYP2C19 have been detected, most of which are defective. Reliable in vitro and in vivo correlations and genotype-phenotype correlations exist, but phenotyping shows an overlap between heterozygous and homozygous EMs. Bridging between ethnic groups is appropriate. Genotyping should minimally include **2* and **3* alleles but should also include **4* to**6* in white subjects (Table III). To decrease the adverse effects of TCAs, doses may need to be decreased in PMs. To increase the efficacy of PPIs, doses may need to be increased in homozygous EMs.

CYP2D6

The simple, inherited EM and PM phenotypes of CYP2D6 that were first observed in sparteine and debrisoquin (INN, debrisoquine) metabolism in 1977 belie a gene of considerable genetic complexity.[36,84,85] The PM phenotype of this enzyme results in large increases of up to 15-fold in the maximum concentration and AUCs of more than 40 therapeutic agents that are primarily metabolized by this route (for a detailed and referenced list, see reference [85a]). These include a number of drugs with a narrow therapeutic range, such as the TCAs and flecainide.[86] They also include a number of drugs in wide use that are of considerable therapeutic value such as the β-blockers metoprolol, timolol, and propranolol. For these drugs, the PM phenotype does result in pharmacodynamic differences in the form of lower heart rates and lower blood pressure at the same dose.[87,88] CYP2D6 is also the primary catalyst for the metabolism of codeine to its active metabolite, morphine,[89] of tramadol to its active metabolite,[90] and of tamoxifen citrate (INN, tamoxifen) to its primary active metabolite, endoxifen.[91]

The unusual properties of the CYP2D6 gene that complicate a simple approach to genotyping to predict activity include the presence of 2 highly homologous pseudogenes adjacent to the coding region on chromosome 10, CYP2D7 and CYP2D8, and the existence of a genetic variant, the *CYP2D6*5* allele,[92] that results in complete removal of the coding sequence. In addition, multiple copies of the entire coding sequence have been described in a number of populations,[93-95] and 13 copies of the gene have been described in a Swedish family.[96] The presence of multiple copies of the CYP2D6 gene within the ge-

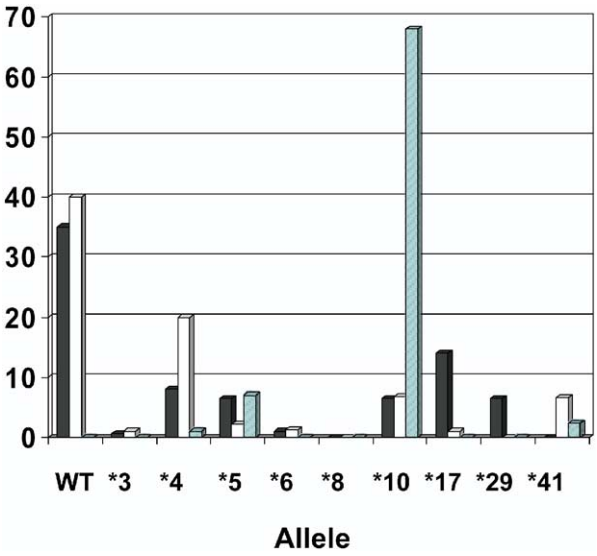


Fig 3. Allelic frequencies of CYP2D6 in black subjects (*solid bars*), white subjects (*open bars*), and Asians (*striped bars*) (adapted from data presented in references 99, 101, 106, and 116). WT, Wild type.

nome of some individuals results in a third phenotype, the ultrarapid metabolizer phenotype.

In vitro–in vivo correlations. A total of 52 alleles of CYP2D6 had been described as of July 2005.[97] Not all of these alleles have clearly defined functional differences from the wild type. It is clear that alleles **3*, **4*, **5*, **6*, **7*, **8*, **11*, **12*, **13*, **14*, **15*, **16*, **18*, **19*, **20*, **21*, **38*, **40*, **42*, and **44* have no activity. In addition, alleles **10*, **17*, **36*, and **41* have substrate-dependent decreased activity. At this time, none of the alleles that are not multiple copies but appear to have increased activity in vitro seem to have increased enzyme activity in vivo.

It is consistent with the high incidence of the ultrarapid metabolizer phenotype in East Africa (13.6%)[97] that multiple copies of the CYP2D6 **1*, **2*, **4*, **9*, and **41* alleles have been reported in African and other populations. The allelic incidence of these high-copy-number alleles varies among populations, with 1.9% in black subjects,[98] 0.5% in Japanese subjects,[99] and 3.3% in Tanzanian subjects.[100]

The relative simplicity of genotype determination has encouraged a large number of investigators to attempt to use genetic approaches to better predict the phenotype of patients within the EM phenotype. To this end, a number of valuable probe drugs that appear to use this metabolic pathway as a primary route of elimination have been described which serve as both in vitro and in vivo probes of activity. These include debrisoquin and sparteine, as described previously, and the widely used probe drugs dextromethorphan[101] and *S*-metoprolol.[102,103]

Which alleles to determine. As illustrated in Fig 3, there are notable differences in the distribution of the most common of these alleles in the 3 main ethnic groups. It is clear that an efficient genotyping strategy for any population of patients or normal volunteers has to take ethnicity into account. Among white subjects, assessment of the most common alleles that result in loss of function would require testing for the **4* allele and should include the **3*, **5*, **6*, and **10* alleles (Table III). In addition, it may be valuable to test for the **41* allele among white subjects, in whom this reduced activity variant is common.[104-106]

In addition, it is likely that an assessment of the *CYP2D6*17* allele,[106] prominent in West African[107] and black populations, would improve the ability of any study designed to predict CYP2D6 metabolism in those populations. Likewise, assessment of the *CYP2D6*10* allele[108-111] should be key to the prediction of the CYP2D6 metabolism phenotype in Asian populations, in whom this allele often has a frequency in the 70% range. It is of note that both the **17* and **10* alleles are not knockout alleles which remove functional CYP2D6 enzyme activity and that their effect on phenotype is, therefore, reduced. As a result, the average rate of metabolism by CYP2D6 is marginally slower in Asian populations, but there is a low incidence (in the 1%-2% range) of the categoric PM phenotype,[101,109] as is the case in African[107] and black[112] populations. Because the **17* allele is the result of a nonsynonymous SNP coding for an area near the active site, substrate-dependent effects have been observed, and a dissociation of the control of debrisoquin, sparteine, and metoprolol metabolism by CYP2D6 in Nigerians has been described.[113]

Clinical relevance. The PM and ultrarapid phenotypes of CYP2D6 differ from EMs by 5- to 15-fold if measured by rates of metabolism or by ratios of parent to metabolite concentrations, and so these represent the 3 most important phenotypes to investigators and clinicians who wish to use CYP2D6 genotyping to predict the clinical effects of drugs. Assessment of the intermediate metabolizer phenotype is difficult because it is quantitatively close to the EM phenotype, there is clear overlap between these 2 phenotypes in every study published, and the change in prescribed dose that might result is, therefore, small.[114] It follows that efforts to predict the ultrarapid metabolizer, EM, or PM phenotypes will be of most clinical value. Strategies to predict clinical outcome that use allele scoring strategies

may also be of value in some populations, as well as in situations where very accurate assessment of phenotype is available to validate any prediction of outcome.[115]

A large number of drugs can be metabolized by CYP2D6. However, the drugs for which this approach will be most valuable will be those that are predominantly metabolized by CYP2D6 or those in which metabolism to important, active metabolites is catalyzed exclusively or primarily by CYP2D6. The clinical value of CYP2D6 genotyping will be most valuable when it provides a significant incremental improvement over what can currently be predicted by use of routinely available clinical tests. As a result, its impact will likely be greatest in oncology and in psychiatry, in which the existing means of predicting effect are limited. It is difficult for care providers who treat patients who are depressed or have bipolar disorder to predict in advance which antidepressants or antipsychotics will work best in which patient, resulting in significant morbidity and mortality rates. Similarly, when the only assessment of the efficacy of antitumor therapy is the return of metastatic disease, it is clear that public health could benefit greatly from better methods of predicting outcome.

Conclusions. Although we have been aware of the CYP2D6 genetic polymorphism for more than a quarter century, it remains the case that there is no situation in which testing for this polymorphism is in routine clinical practice. That being said, CYP2D6 genotyping is now an established and frequently used tool in drug development that is of great value in the determination of the effects of this important polymorphism on the pharmacokinetics of new molecular entities which undergo metabolism by the enzyme. In addition, FDA-approved testing is now available, an increasing number of companies provide CYP2D6 genotyping under Good Laboratory Practice conditions, and an increasing number of medical centers provide this service to patients under their care. It will be increasingly important for physicians, pharmacists, and other care providers to be able to provide coherent therapeutic recommendations to patients with predetermined pharmacogenetic data.

UGT1A1

UGT1A1 has been shown to metabolize various drugs,[37] including SN-38, the active metabolite of irinotecan,[117] a cytotoxic agent approved for metastatic colorectal cancer usually administered in combination with 5-fluorouracil. (It is also commonly used off-label for other solid tumors.) Its use is limited by toxicity, including life-threatening neutropenia and associated infection, most common on the every-3-week schedule. The other major toxicity, more problematic when it occurs, is severe or life-threatening diarrhea, necessitating either parenteral fluids or hospitalization (or both), which occurs more on the weekly schedule.

Irinotecan's disposition in humans is complex.[118] The parent drug is inactive. A fraction is metabolized by CYP3A4 and CYP3A5 (the latter being minor) to inactive metabolite(s). It is hydrolyzed by carboxylesterases to SN-38, the active form. SN-38 is further metabolized by glucuronosyltransferases, primarily by UGT1A1. In addition, SN-38 is a substrate for UGT1A9, UGT1A6, UGT1A7, and UGT1A10, although the clinical significance of variability in these other enzymes is not clear at this time.

Which alleles to determine. In patients treated with irinotecan (weekly schedule), diarrhea appeared to correlate with decreased glucuronidation.[119] Other studies have shown that neutropenia (with the every-3-week schedule) is correlated with the *UGT1A1*28* genotype. Table IV provides a list of adequately sized studies evaluating *UGT1A1*28* and irinotecan toxicity or pharmacokinetics.

*UGT1A1*6*, which only has 30% of the wild-type activity, is consistently associated with neonatal hyperbilirubinemia in Asians. In one study in 85 Japanese subjects, **6* showed 90% effect of **28*,[120] whereas in another study in 118 Japanese subjects, no significant correlation between genotype and toxicity was observed.[121] *UGT1A1*60*, which shows a higher prevalence in African populations (32% as compared with 14% in Asians or 9% in Europeans), is associated with irinotecan pharmacokinetics and bilirubin levels in univariate analysis but not multivariate analysis.[120] In a second study, *UGT1A1*60* did not show a correlation with either irinotecan pharmacokinetics or toxicity.[122]

Conclusions. In summary, the existing data indicate that *UGT1A1*28* is a valid biomarker (distinguishing 3 genotypes) for decreased UGT1A1 activity and for increased irinotecan toxicity and should be measured (Table III) along with other clinical measures (eg, bilirubin levels) in treating patients taking irinotecan. Additional prospective studies with the *UGT1A1*6* genotype should be considered in Asian populations to determine its association with irinotecan toxicity. *UGT1A1*60* should be evaluated further, particularly in African populations, in which it has the highest frequency. In addition, the role of various transporters (multidrug resistance protein 2, organic anion transporting polypeptide 1B1) in the disposition of irinotecan will require further evaluation to better define variability in irinotecan toxicity.

Table IV. Summary of adequately sized studies of *UGT1A1*28* and irinotecan/SN-38 pharmacokinetics and metabolism or toxicity[120-129]

Study type	*Measured parameters*	*Outcome*	*Reference*
In vitro study with 44 microsomes	Glucuronidation rate	6/6 < 6/7 < 7/7	129
Case-control study in 118 Japanese subjects	Toxicity	7/7 higher risk for grade 4 leukopenia or diarrhea than 6/7 and 6/6	121
Prospective clinical study in 20 Asians	Neutropenia	ANC nadir 7/7 2.5-fold lower than 6/6	123
	AUC	AUC 7/7 3.9-fold higher than 6/6	
Pharmacokinetic study in 65 Europeans (58 genotyped)	AUC (SN-38G/ SN-38)	No significant correlation	124
Prospective clinical study in 51 Spaniards (with docetaxel)	Toxicity	No genotype-dependent differences in toxicity	125
Prospective study in 66 Americans	Neutropenia	7/7 higher risk (9.3-fold) in grade 4 leukopenia	122
	AUC	7/7 lower AUC (1.8-fold) than 6/6	
Pharmacokinetic study in 94 Europeans	AUC (SN-38G/ SN-38)	6/6 < 6/7, 7/7	126
Pharmacokinetic study in 85 Japanese subjects (41 genotyped)	PK (SN-38G/ SN-38)	6/6 < 6/7 < 7/7	120
Clinical study in 75 Europeans (with 5-fluorouracil)	Neutropenia	7/7 < 6/7 < 6/6	127
	Diarrhea	No significant genotype-dependent correlation	
Clinical study in 75 Spaniards	Diarrhea	7/7 < 6/7 < 6/6	128

ANC, Absolute neutrophil count; SN-38G, glucuronide of SN-38; PK, pharmacokinetics.

CYP3A

Safe and efficacious treatment with CYP3A substrates is sometimes hampered by the substantial degree of variability in hepatic and intestinal enzyme activity that exists in the human population.[130,131] Previous investigators have attributed much of the variability in basal or constitutive CYP3A activity to genetic sources.[132] However, the search for mutations in the major functional *CYP3A* genes (*CYP3A4*, *CYP3A5*, and *CYP3A7*) that have a significant effect on oral drug bioavailability or systemic clearance has yielded mixed results.

CYP3A4 genotypes. Much of the effort has been focused on CYP3A4 because of the dominant role that it plays in drug elimination. Numerous allelic variants of *CYP3A4* have been reported to the CYP allele Web site.[133] One of the most common, *CYP3A4*1B*, represents a single base substitution in a putative response element (NFSE) found in the 5′-flanking region of the gene. Some studies have linked this allelic variant to altered gene transcription and enzyme activity[134,135]; however, other groups have failed to find a clear association at the in vitro[136,137] and in vivo level.[138-140] Interpretation of the *CYP3A4* genotype-phenotype activity data is complicated by the presence of linkage disequilibrium between the *CYP3A4*1B* allele and other SNPs found in the CYP3A gene locus, including a functionally significant mutation in the *CYP3A5* gene (*CYP3A5*3*).[141]

A number of *CYP3A4* coding mutations that result in changes to the enzyme structure have also been discovered, and some (*CYP3A4*2*, *CYP3A4*17*, *CYP3A4*18*) appear to affect catalytic function in vitro.[132] However, the frequency of these variations is relatively low, and thus the association with altered in vivo metabolic function remains undetermined.

CYP3A7 genotypes. Although the expression of CYP3A7 in humans is repressed after birth[142] and its level in the adult liver is much less than that of CYP3A4,[143] an unusual mutation in its 5′-flanking region may confer significant expression in some individuals. A swap of 60 base pairs that includes the proximal pregnane X receptor response element of the *CYP3A4* gene into the *CYP3A7* gene (comprising 6 base substitutions) has been associated with higher levels of *CYP3A7* messenger ribonucleic acid in both the liver[141,144] and the small intestine.[144] The frequency

of the variant allele is relatively low (2% in white subjects and 6% in black subjects), but it may contribute to an extreme phenotype with a higher than average metabolic clearance of CYP3A substrates that has been seen in large populations.[131]

CYP3A5 genotypes. Only a fraction (approximately 10%-25%) of livers from donors with white European ancestry express a level of CYP3A5 protein that can be readily detected by Western blot analysis.[145] The primary genetic basis for this distinctive phenotype is the result of a single SNP found in intron 3 of the *CYP3A5* gene that causes aberrant messenger ribonucleic acid splicing and predicted truncation of the CYP3A5 protein. Additional mutations that are more common in populations of African ancestry (*CYP3A5*6* and *CYP3A5*7*) can also contribute to the low-expression phenotype.[141,146] A larger percentage of Africans and black subjects express the CYP3A5 enzyme (45%-55%) than do white subjects, whereas the frequency in Chinese subjects is predicted to fall in between.[131]

The presence of CYP3A5 in the hepatic and intestinal microsomal fractions has been associated with increased metabolic activity toward some but not all CYP3A4 substrates.[147] Tissues from donors with 1 or 2 copies of the wild-type *CYP3A5*1* allele exhibit an intrinsic clearance that can be 25% to 100% higher than that from donors homozygous for the *CYP3A5*3* allele, although considerable interindividual variability unrelated to the *CYP3A5* genotype exists. The most convincing evidence to suggest that the *CYP3A5*3* mutation affects drug disposition in vivo comes from studies of tacrolimus kinetics in organ transplant patients. Multiple groups of investigators have found that individuals carrying the *CYP3A5*1* allele have lower trough blood concentrations (normalized for dose) than do *CYP3A5*3* homozygotes.[149-152] The simplest interpretation of the data is that patients with a functional *CYP3A5* allele have a higher capacity to metabolize tacrolimus, a known CYP3A5 substrate,[153] and that this necessitates a higher oral dose to achieve blood levels within a targeted therapeutic range. However, because therapeutic blood level monitoring is performed routinely in these patients to contend with the variability of genetic and nongenetic (eg, induction, inhibition, pathophysiologic) origins, it is not clear whether genetic testing will prove to be necessary. Given that patients with the *CYP3A5*1* allele were more likely to require a longer time after transplantation to have a therapeutic blood concentration than patients homozygous for the *CYP3A5*3* allele,[154] a prospective genetic test could reduce the time needed to attain a stable and effective blood concentration, resulting in a favorable cost/benefit ratio.

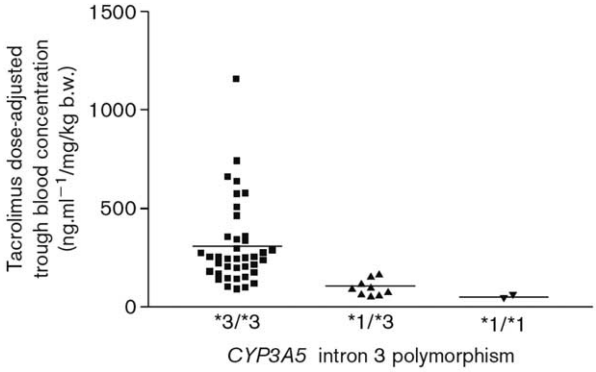


Fig 4. Dose-adjusted trough blood concentration of tacrolimus (in nanograms per milliliter per milligram per kilogram body weight) according to CYP3A5 intron 3 polymorphism, G6986A (*CYP3A5*3/*3*, n = 39; *CYP3A5*1/*3*, n = 9; and *CYP3A5*1/*1*, n = 2). Blood concentrations are adjusted to the last dose. The mean values are indicated. (Reprinted with permission from Haufroid V, Mourad M, Van Kerckhove V, Wawrzyniak J, De Meyer M, Eddour DC, et al. The effect of CYP3A5 and MDR1 [ABCB1] polymorphisms on cyclosporine and tacrolimus dose requirements and trough blood levels in stable renal transplant patients. Pharmacogenetics 2004;14: 147-54.)

The effect of the *CYP3A5*3* polymorphism on the disposition of other CYP3A substrates has been less conclusive. For example, with respect to the CYP3A probe substrate midazolam, some investigators have reported the predicted higher metabolic phenotype for individuals carrying the *CYP3A5*1* allele,[155,156] but others have not.[157-159] It is not clear what distinguishes midazolam from tacrolimus, because both drugs are excellent substrates for CYP3A5 and CYP3A4.[148,153] It is possible there are additional factors yet to be described that affect the in vivo disposition of one substrate but not the other. For example, Katz et al[160] have suggested that saturation of intestinal CYP3A4 activity may be a necessary prerequisite for the CYP3A5 phenotype to become apparent.

In summary, although genetic mutations in the CYP3A genes can affect the metabolic fate of clinically important drugs, it remains to be seen whether genetic testing will prove to be a cost-effective approach to individualizing drug therapy. Of the variant alleles that have been characterized to date, mutations in the *CYP3A5* gene appear to be the most promising (Fig 4).

ABCB1 (MDR1)

Until recently, the role of transporters in drug disposition and response received limited attention relative to

that of drug-metabolizing enzymes.[161] The *ABCB1* (multidrug resistance 1 [*MDR1*]) gene product P-glycoprotein is the most widely studied drug transporter and has a recognized role in the bioavailability and biliary, intestinal, and renal excretion of numerous drugs.[162-164] P-glycoprotein is also a major component of the blood-brain, blood-testes, and maternal-fetal barriers and limits both therapeutic and toxic responses of drugs in the central nervous system, testes, and fetus. Given the broad substrate specificity of P-glycoprotein, variability in the expression or function of this transporter is predicted to have a significant impact on drug disposition and response.

Genetic variation in *ABCB1* has been reported by numerous groups, and more than 50 variant sites are listed at the Pharmacogenetics and Pharmacogenomics Knowledge Base Web site.[165-170a] Common coding region variants have been reported for the coding region and 5′-untranslated region,[165,170] and there are significant differences in allele frequencies across various ethnic groups. For example, the nonsynonymous 2677G<T allele is found at a frequency of 40% to 46% in white subjects, Asian Americans, and Mexican Americans and only 10% in black subjects. In contrast, 3421T<A is an African American–specific variant found at a frequency of 11% in this population.

Investigation of the functional consequences of P-glycoprotein coding SNPs suggests that the common Ala893Ser variant has little effect on transporter function. Unfortunately, no validated assay for measuring P-glycoprotein function exists, and variability in cell culture models and transport assays makes these data difficult to interpret. In a single report the 893Ser variant of P-glycoprotein showed a significant increase in function by use of digoxin as a substrate.[170] However, with the use of a variety of substrates, other investigators have failed to find any difference in function between the reference and 893Ser variant of P-glycoprotein.[164,171,172] To date, there are no functional data on the common African American Ser1141Thr variant of P-glycoprotein.

Despite the lack of functional data supporting differences in transporter function for P-glycoprotein coding region variants, numerous clinical studies have been reported on the association of *ABCB1* SNPs with pharmacokinetic, pharmacodynamic, and disease risk endpoints.[162,163] An initial report by Hoffmeyer et al[168] in 2000 provided evidence that white subjects who are homozygous for the synonymous 3435C<T variant have decreased levels of intestinal P-glycoprotein and a corresponding increase in digoxin maximum plasma concentration values. However, follow-up studies have been inconclusive, with increased,[173-175] decreased,[176,177] and unchanged[178,179] digoxin levels being reported for individuals with the 3435TT genotype. Similar discordant data exist on the effect of *ABCB1* genotype on the pharmacokinetics of fexofenadine,[170,180] nelfinavir,[181,182] cyclosporine (INN, ciclosporin),[183-193] and tacrolimus.[183,194-199] An intriguing association between *ABCB1* SNPs and drug-resistant epilepsy has recently been reported; however, these findings have not been confirmed in all subsequent studies.[190-203] Clearly, further investigation is needed to define the impact, if any, of *ABCB1* polymorphisms on drug disposition and response.

In summary, despite extensive investigation during the last 4 years, there is a lack of evidence supporting a clear association between *ABCB1* genotype and clinical drug response or toxicity. Several hurdles related to the in vitro and in vivo study of transporter function need to be overcome before this field will advance. In contrast to drug metabolism, the study of drug transporters is limited by the availability of specific and sensitive transporter substrates and inhibitors. Whereas digoxin and fexofenadine are two of the best-characterized P-glycoprotein substrates, there is no single pharmacokinetic parameter that robustly reflects transporter function. This is one possible explanation for the lack of an in vivo–in vitro correlation for P-glycoprotein activity. Well-validated systems for the functional analysis of transporters are also not available, making it difficult to correlate changes in deoxyribonucleic acid sequence with alterations in transporter function. The haplotype structure of *ABCB1* is complex, and consideration of haplotypes instead of single SNPs is likely to more accurately reflect transporter function.[165,169,202,204,205] Therefore collection of *ABCB1* genotype-haplotype information in drug development studies is currently considered exploratory. An ongoing analysis of genotype-haplotype data coupled to pharmacokinetic, pharmacodynamic, and toxicologic phenotypes collected during drug development will further our understanding of the importance of genetic variation in *ABCB1* and other drug transporters in determining variability in drug response.

DISCOVERY OF NEW PHARMACOGENETIC VARIANTS DURING DRUG DEVELOPMENT

The use of pharmacogenetics in IND and NDA submissions has until now focused largely on “known valid” or “probable valid” biomarkers.[206] The use of pharmacogenetics in this way requires little or no novel discovery of gene variants that influence drug response. There are many indications, however, that in the near

future applications of pharmacogenetics in drug development will include an increasing emphasis on explicit efforts either to refine probable or exploratory biomarkers or to identify novel ones.

There are a number of contexts in which this can be expected to occur. In the simplest case this may take the form of refining exploratory biomarkers already suggested by earlier work, for example, variants in the *ABCB1* gene.[163] If there were reason to be concerned about the role of variation in this gene, it would not be sufficient to show no association with 3435C<T and not consider variation in the gene any further. Similarly, it would be equally insufficient to find association with the 3435C<T variant and then propose it as diagnostic for drug response because this polymorphism may not be causal. If a marker is used diagnostically instead of the causal variant, there is every reason to believe that it would not work consistently across ethnic or racial groups because of varying patterns of linkage disequilibrium. The example of situations such as CYP2D6, in which the simple approach is often justified (eg, checking for association with null or reduced activity genotypes), should not inform the approach taken in situations in which our knowledge of the role of variation in the relevant gene is very limited and quite possibly incorrect. Instead, these situations require a systematic discovery effort. At the next level of complexity, we can imagine situations in which a variable response to a compound has been observed but relevant valid or even probable biomarkers are not already known.

Finally, an argument could be made that some straightforward discovery efforts should be carried out even if the observed pattern of variation in a phase III trial did not present a compelling case for pharmacogenetic investigation. Just because genetics is not needed for drug development or approval, it does not follow that genetics is irrelevant in the effort to clinically optimize the use of the medicine. The strict inclusion and exclusion criteria of trials, as well as the detailed response information normally collected, present important opportunities for research that often are not easily recapitulated once a drug goes to market.

For these reasons, we may assume that pharmacogenetics during drug development will include an increasing effort to discover new variants. This raises the question of how such discovery will be carried out, as well as how it will be interpreted. The first question is whether to focus on candidate genes (and pathways) or genome-wide analyses, which are slowly becoming feasible. Although genome-wide approaches will have their uses, it is possible to make a strong case that the obvious candidate genes, such as the drug target (and associated pathway) and genes encoding the major metabolizing enzymes, as well as transporters, should be investigated as a priority for all prescription medicines.[207]

As one of many possible examples illustrating candidate gene approaches in pharmacogenetics, Tate et al[208] looked for genetic contributors to dose requirements of the antiepileptic drugs carbamazepine and phenytoin. The phenotype considered was the maximum exposed dose during the regular clinical use of the 2 drugs. To identify gene variants associated with dose, Tate et al looked at low-activity variants in *CYP2C9* and the 3435 variant in the *ABCB1* gene. They also considered the *SCN1A* gene, one of the genes encoding the α-subunit of the voltage-gated sodium channel, the target of both drugs. No common functional variation was then known in the gene, so the authors used a haplotype-tagging approach.[208,209]

The *CYP2C9*3* allele was found to be significantly associated with the maximum exposed dose of phenytoin, whereas one of the SNPs selected as a tag was found to be significantly associated with the maximum exposed dose for both carbamazepine and phenytoin. This SNP was then shown to fall in the penultimate site of the consensus sequence for the 5′ splice donor site of an alternative (presumptively neonate) form of exon 5 not previously recognized. This example illustrates that detailed haplotype analyses of obvious candidate genes are likely to identify gene variants relevant to drug response and increases the case for more discoveries during the development of new medicines.

Whether the approach is based on candidate genes or, ultimately, the whole genome, there are 2 methods that may be used for comprehensive efforts to identify new gene variants which are associated with drug responses, often referred to as direct (or sequence-based) and indirect (or map-based).[210] In the direct approach exhaustive discovery of variants is performed in genes or gene regions of particular relevance (eg, exons of the target of the drug, relevant transporters) in either all or a subset of the relevant individuals. In the indirect approach a set of variants are selected in the gene to represent other variants through linkage disequilibrium or the nonrandom association of alleles at different loci. If the focus is on well-known polymorphisms as opposed to one of these more comprehensive approaches, the study should be referred to as candidate polymorphism instead of candidate gene, which implies that variation in the gene has been comprehensively assessed (in some fashion).

Linkage disequilibrium mapping has been extensively reviewed,[211,212] and we will not reprise those details here. Instead, we note a number of points of particular relevance in pharmacogenetics. First, haplotype mapping methods should now be viewed as suf-

ficient to represent common variation in genes of interest by use of tagging SNPs selected in a reference resource such as that provided by the HapMap Project.[213] As a rough indication of the efficiency that may be expected, Ahmadi et al[214] considered 2100 kilobases of sequence composed of 55 important drug-metabolizing enzymes. It was found that only 179 and 156 tagging SNPs are sufficient to represent all of the common variations in these genes in European and Japanese population samples, respectively, with a very modest loss of power in comparison with direct assay of all common SNPs in the region (SNPs with minor allele frequency greater than approximately 0.05). When it is noted that there are 4000 such common SNPs predicted in relevant sequence intervals, the magnitude of the economy provided by haplotype tagging becomes readily apparent.

The important qualifications here are that (1) rare variants are unlikely to be well represented and (2) the ethnic structure of phase III populations will need to be taken into account, because tagging SNPs do not transfer directly across population or ethnic groups. When Ahmadi et al[214] tested their tagging SNPs in an independent population sample (ie, individuals drawn from Aberdeen, Scotland, for tags selected in North Europeans as represented in CEPH [The Foundation Jean Dausset-Centre d'Etude du Polymorphisme Humain]), it was found that all common SNPs were well represented. The SNPs with lower minor allele frequencies, however, were very poorly represented. This would imply that it may often be necessary to resequence genes of particular significance.

Complications related to ethnic or racial diversity may prove less of a long-term concern. For example, Ahmadi et al[214] selected a set of SNPs that would perform well in both the Japanese and the European population samples. They found that only a modest increase was required to define a set which would work well across individuals drawn from both groups. Given the rapidity with which many decisions need to be made during the drug development process, there may be an argument for the creation of panels of such "cosmopolitan" tagging SNPs for genes of particular pharmacologic significance. This way the panel could simply be applied as opposed to being tailored to the particular makeup of each trial population.

SUMMARY

Pharmacogenomic data can facilitate our understanding of the sources of variability in drug response and can potentially lead to improved safety and efficacy of drug therapy for individual patients. Through various initiatives,[4,5] the FDA is encouraging drug developers to apply the rapidly evolving pharmacogenomic tools and integrate these data into the evaluation of patient variability. The FDA has clarified when these data are required submissions and when they are exploratory data that can be shared via a newly established process (voluntary genomic submission).[6,7]

Increasingly, pharmacogenetics and genomic information are being included in drug labeling before market approval (eg, trastuzumab [Herceptin; Genentech Inc, South San Francisco, Calif], atomoxetine [Strattera], and voriconazole [Vfend])[21] or after approval, when new information becomes available (eg, thioridazine [Mellaril], 6-mercaptopurine [Purinethol], and irinotecan [Camptosar]),[21] so that health care providers and patients have updated information on how genomics, along with other factors (age, gender, hepatic, renal impairment, concomitant medications, and others), can influence individual responses. Various genomic tests are being developed for use with the previously mentioned or other drug products. For example, a recently approved chip provides genotyping of *CYP2D6* and *CYP2C19*.[217] Another test was approved to provide genotyping of *UGT1A1*.[218]

There are many challenges to the effective translation of pharmacogenomic information to clinical practice, and they need to be addressed before the full potential of pharmacogenomics to optimize patient therapy can be realized. This commentary addresses one of the critical issues in the collection of pharmacogenomic data. Defining what basic polymorphic alleles to evaluate in various ethnic or racial groups is important for common, known valid metabolic biomarkers such as *CYP2D6*, *CYP2C9*, *CYP2C19*, and *UGT1A1* (Table III). It is also timely to discuss emerging data for exploratory biomarkers (eg, *CYP3A4/3A5*, *ABCB1*, or methods involving tagging SNPs) because their correlations with clinical response have been increasingly evaluated during drug development.

Other clear challenges include the following: education of health care providers and patients, insurance coverage of pharmacogenomic tests, the availability of robust and valid tests, and the need for an interdisciplinary counseling team approach to address complex issues with individual patients. Many individuals and organizations are working to remove these barriers to full use of pharmacogenomics to improve public health.

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the company. Dr Anderson is an employee of AstraZeneca and holds stock options in the company.

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