PhRMA White Paper on ADME Pharmacogenomics

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Pharmacogenomic (PGx) research on the absorption, distribution, metabolism, and excretion (ADME) properties of drugs has begun to have impact for both drug development and utilization. To provide a cross-industry perspective on the utility of ADME PGx, the Pharmaceutical Research and Manufacturers of America (PhRMA) conducted a survey of major pharmaceutical companies on their PGx practices and applications during 2003-2005. This white paper summarizes and interprets the results of the survey, highlights the contributions and applications of PGx by industrial scientists as reflected by original research publications, and discusses changes in drug labels that improve drug utilization by inclusion of PGx information. In addition, the paper includes a brief review on the clinically relevant genetic variants of drug-metabolizing enzymes and transporters most relevant to the pharmaceutical industry.

Keywords: *Pharmacogenomics; ADME; PhRMA; drug-metabolizing enzymes; genotyping*

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The objective of this white paper is to present a pharmaceutical industry perspective on the utility of pharmacogenomics (PGx) related to the absorption, distribution, metabolism, and excretion (ADME) properties of drugs for drug development and utilization. This paper is not intended to provide best practices for ADME PGx in drug development or utilization or to address ethical ramifications of ADME PGx, but it does offer perspectives on the current state of practices, strategies, knowledge, and key information gaps that need to be addressed to fulfill the promise of personalized medicine.

Improved ADME PGx understanding has led to an evolution in how polymorphic ADME genes are considered during drug development and utilization. Only a decade ago, concerns related to wide pharmacokinetic variability were frequently the basis for decisions not to develop substrates of polymorphic drug-metabolizing enzymes (DMEs), such as cytochrome P450 (CYP) 2D6. In contrast, recent integration of

ADME PGx in drug development is highlighted by the approval and launch of atomoxetine, a sensitive CYP2D6 substrate. This change in position concerning polymorphic DMEs is driven by application of PGx, guidances for industry from each of the 3 major drug regulatory agencies, and drug label text regarding PGx testing.

THE PHARMACEUTICAL RESEARCH AND MANUFACTURERS OF AMERICA PGx SURVEY

A series of questions were developed to elicit broad information about current ADME PGx practices in large pharmaceutical companies. Survey respondents were instructed to base all answers on clinical trials that were initiated during the period 2003-2005. Most of the questions were multiple choice to facilitate consistency, compilation, and reporting of responses. Companies were also asked to provide citations for peer-reviewed original PGx ADME research published by industry scientists (up to 3 papers per company) and to provide masked examples of how PGx ADME information has been used for internal decision making (1 per company) and regulatory interactions (1 per company). Pharmaceutical Research and Manufacturers of America (PhRMA) lawyers reviewed the survey to help ensure that it did not solicit competitive information or could form a basis for collusion. Subsequently, PhRMA staff solicited and compiled the responses from 14 PhRMA member companies. To preserve anonymity, the authors received only aggregated data, except for citations of published papers. Not every company answered every question. The multiple-choice questions and aggregated results are presented in the appendix and summarized below.

Recent ADME PGx Practices in Drug Development

Integration of ADME PGx Samples and Genotyping

Collection of DNA samples with appropriate informed consent for ADME PGx testing is standard industry practice for clinical studies in which intensive pharmacokinetic data are collected (question 1; Figure 1). However, there is greater heterogeneity across companies when considering whether sample collection is a "required or optional" study activity (question 11; Figure 2). In phase I studies, most companies sometimes integrate ADME PGx as required study activity and sometimes make it an optional activity for individual subjects. Participation in ADME PGx research is typically an optional activity in phase II and III studies. Most or nearly all companies usually collect these samples in phase I studies (eg, first in human, multiple rising dose, drug-drug interaction). The most uniform sample collection is in multiple rising-dose studies, although there is the heterogeneity of practices for special population studies, which may reflect the particular mix of these studies conducted by different companies during the survey period. For example, DNA collection might be less frequent in pediatric studies compared with hepatic impairment studies. Collection of DNA samples from every study type is not prevalent, likely reflecting that most companies recognize that PGx testing is not useful in some studies (eg, those testing biologicals rather than small molecules). That collection of DNA samples for ADME PGx testing is less consistently obtained in phase II and phase III studies is not surprising, as these studies often collect limited or no pharmacokinetic data, PGx analysis can be performed using subsets of samples from these larger studies, or that the potential benefits of ADME PGx research in late studies can be outweighed by a perceived impact on study enrollment rates. Nonetheless, a majority of companies find that there is utility at times to collect PGx samples in larger clinical studies, as polymorphisms in ADME genes can also have consequences for safety and efficacy.

Although DNA sample collection for ADME PGx testing is the usual practice, the samples are not always used (question 2; Figure 3). This reflects that, after the results of many studies become known, it is apparent that ADME PGx testing cannot further aid in the interpretation of the findings. In a precautionary, proactive manner, clinical trialists continue to collect samples because one does not know a priori whether they will be needed, and it is typically very difficult to go back to trial subjects to obtain samples and consent after utility has emerged. Every company at least sometimes performs ADME PGx testing in first-in-human and multiple rising-dose studies, whereas the majority of companies at least sometimes perform ADME PGx testing in every other study type (eg, drug-drug interaction, pivotal).

Every company at least sometimes uses an ADME-related genotype as a study design element (questions 3a/b and 4). A protocol-specified ADME PGx analysis plan is the most prevalent application. This may include stratification of trial results according to genotype or use of genotype as a factor in statistical models. Uses of an ADME-related genotype for

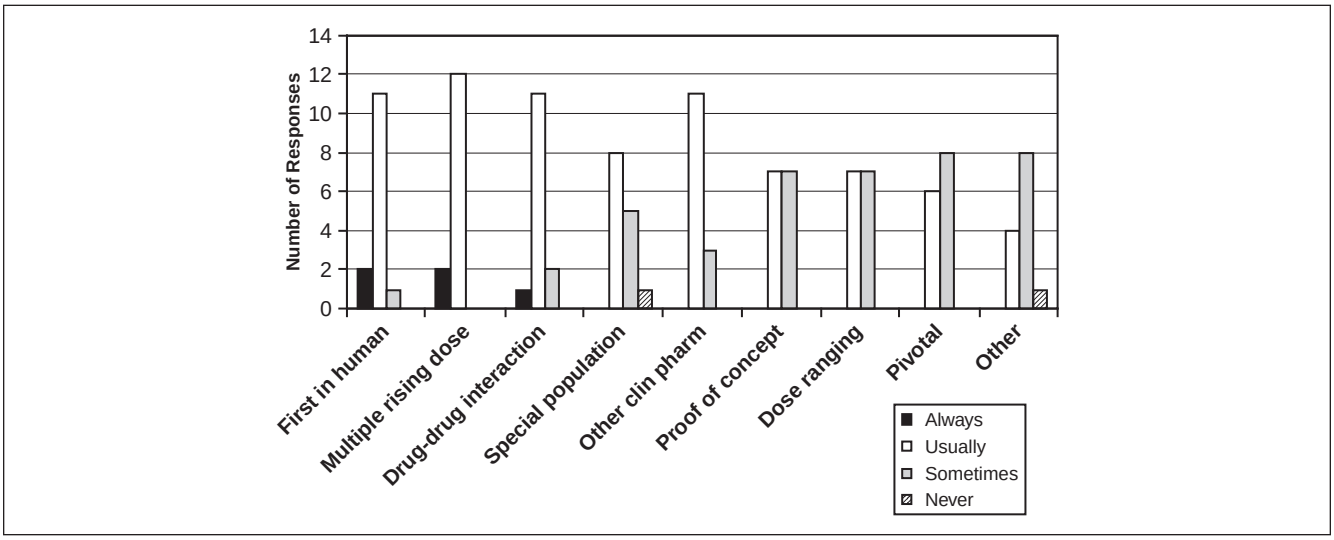

Figure 1. How often has your company collected DNA with consent for ADME-related genotyping in clinical studies?

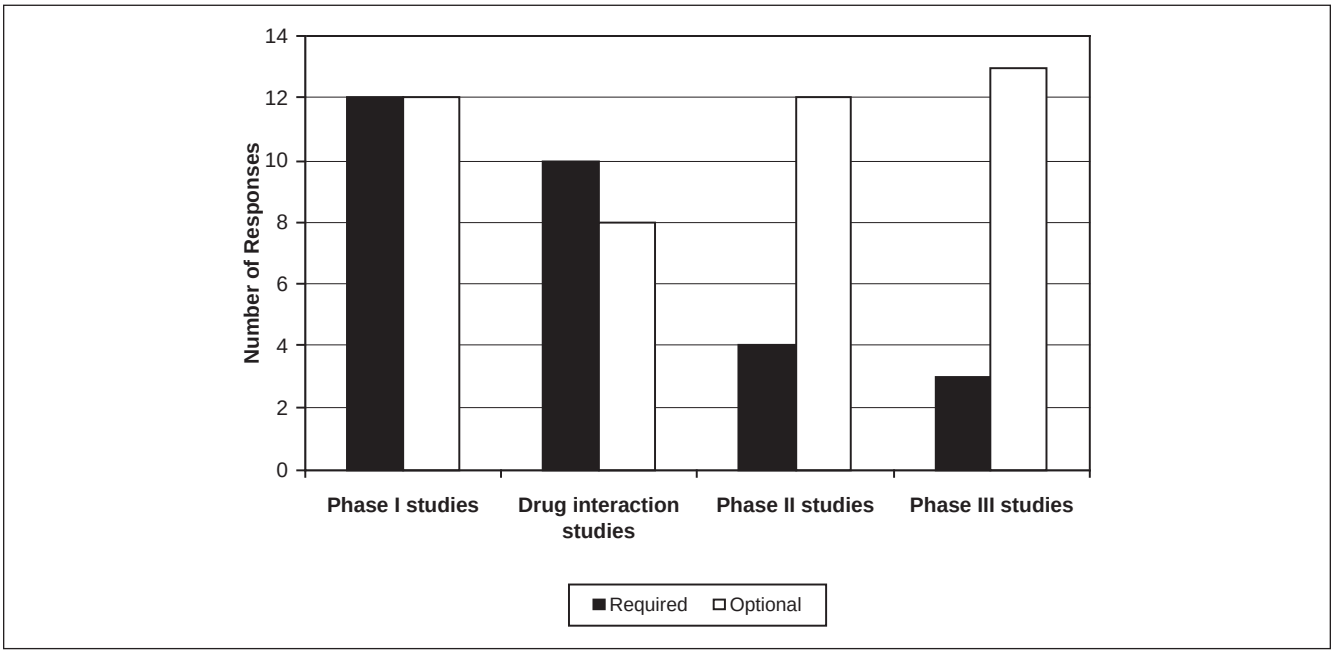

Figure 2. When ADME PGx research has been included in a trial, has it been a required study activity or optional for each subject? (It was acceptable for the respondent to check both required and optional boxes in the survey response.)

either subject selection (inclusion criterion) or screening (exclusion criterion) are equally prevalent and used by most companies. Many of these applications have been based on genes categorized as known valid biomarkers (eg, CYP2C9, CYP2D6, thiopurine methyltransferase [TMPT], uridine glucuronosyltransferase [UGT] 1A1) by the US Food and Drug Administration (FDA) (http://www.fda.gov/cder/genomics/genomic_biomarkers_table.htm), although 1 company reported to have used CYP3A5, methylguanine methyltransferase (MGMT), or glutathione-S-transferase M1 (GSTM1) genotype as either a subject selection or screening criterion.

Nearly every company has at least sometimes written an ADME PGx plan for a compound in development (question 7). This indicates that prospective

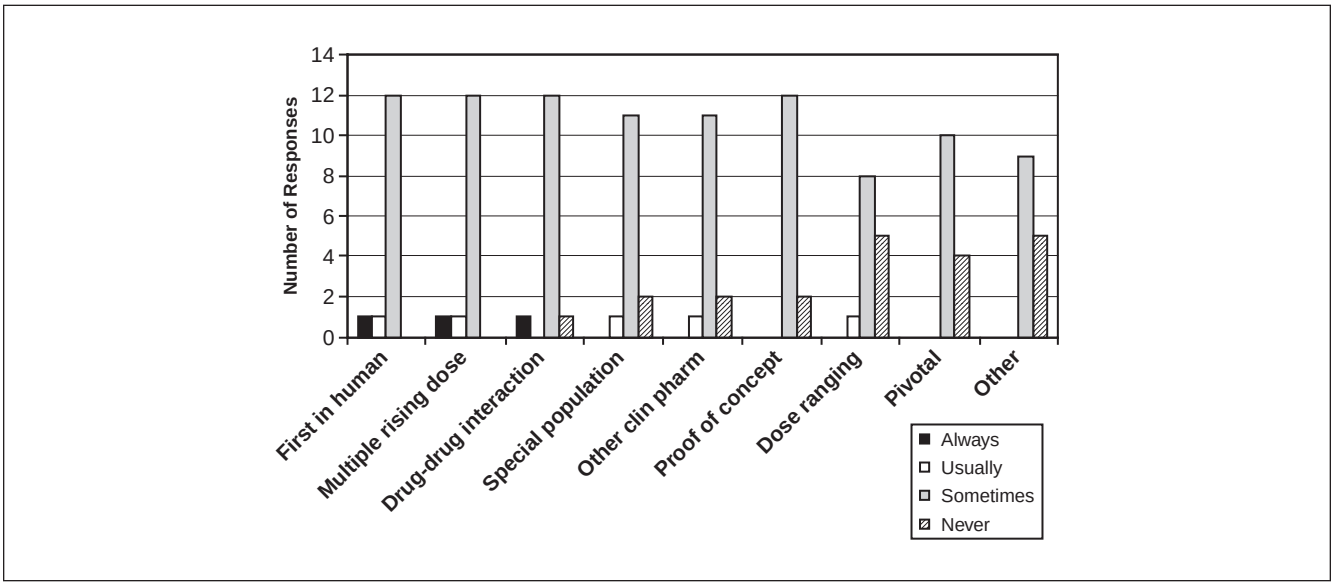

Figure 3. How often has your company performed ADME-related genotyping in clinical studies?

consideration of ADME PGx as an important element of drug development is becoming more prevalent; however, this is a usual practice at only a minority of companies (3/14). It is not surprising that this more formal integration of ADME PGx in drug development is lagging compared with the other forms of ADME PGx activities. Nonetheless, it is clear that the recent level of ADME PGx activity in the pharmaceutical industry is increasing.

Laboratory Practices

All, or nearly all companies test for variants in genes for 6 well-known polymorphic DMEs—CYP2D6, CYP2C9, CYP2C19, CYP3A5, CYP3A4, and UGT1A1 (question 5; Figure 4). All of these except CYP3A4 and 3A5 have been categorized as known valid biomarkers by the FDA. Genotyping of TMPT is not prevalent in the pharmaceutical industry, probably reflecting its limited substrate range. Polymorphisms in drug transport proteins are assayed by a majority of companies. The most commonly genotyped transporters are multidrug resistance 1 (MDR1, also known as P-glycoprotein or ABCB1), breast cancer resistance protein (BCRP, also known as ABCG2), and organic anion-transporting polypeptide 1B1 (OATP1B1, also known as OATP-C or SLCO1B1). Genotyping of other DMEs and other ADME-related genes is prevalent but not uniform across the pharmaceutical industry. It is not a prevalent practice to use a biochemical assay to confirm the veracity of a genotype-predicted phenotype (question 12: eg, making sure that someone with an extensive metabolizer genotype is not actually a poor metabolizer because he or she has ingested an enzyme inhibitor). Possible reasons that this is not done more frequently include sufficient confidence that the presence of a small number of phenocopies would not alter the decisions made on PGx results and the use of exclusion criteria to limit exposure to such agents.

Two main approaches for selecting which ADME genes to study in PGx research are prevalent in the pharmaceutical industry (questions 6 and 16). The first approach is guided by preclinical drug metabolism findings. It proposes a threshold that predicted metabolism by a genetically polymorphic DME is at least 30% of total metabolism of a study drug. When this criterion is met, genotyping for that DME is implemented in clinical studies. In addition, when there is precedence for metabolism-based toxicity, for example, owing to structural similarity, a study drug may be subjected to more extensive preclinical ADME PGx analysis. The second approach is to assess the influence of a broad range of polymorphic ADME-related genes on the clinical pharmacokinetics of a study drug, regardless of whether there is prior knowledge that the gene product is involved in the disposition of the study drug.[35] To facilitate this second approach, high-throughput platforms that enable simultaneous assay of variants in essentially all known common ADME-related genes have been

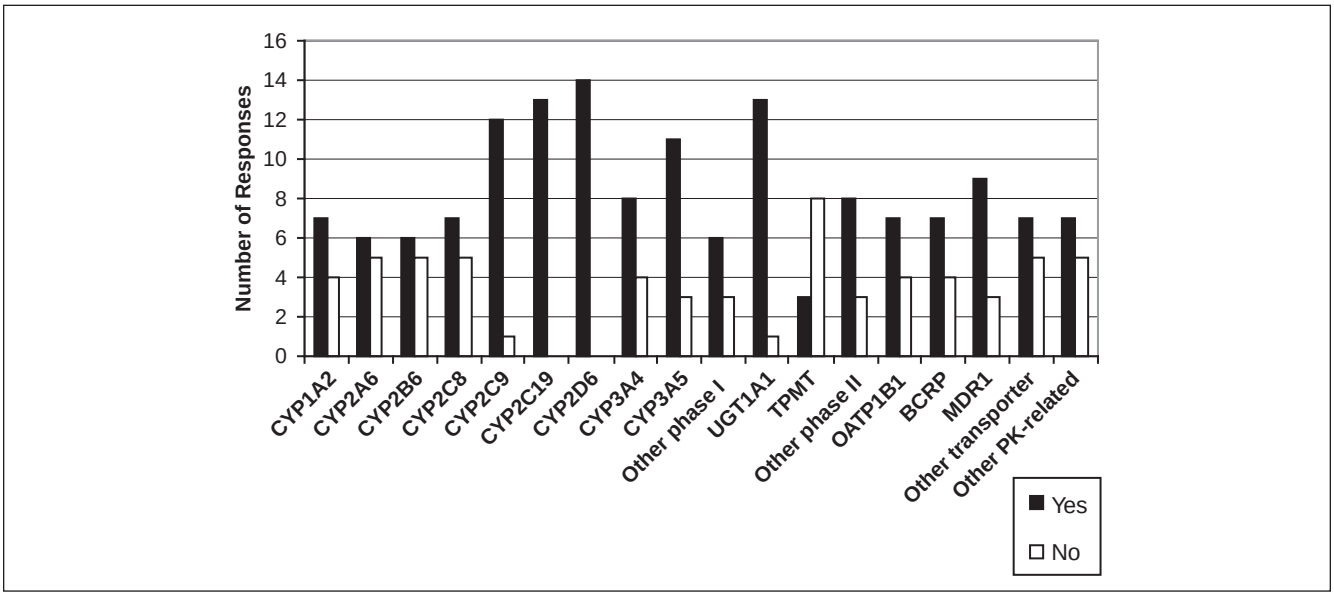

Figure 4. Breadth of genotyping: What genes are currently genotyped?

developed; however, the use of these platforms is not prevalent, although several companies have used them (6/14).

Nearly all companies have internal PGx laboratories performing genotyping (question 8). Nonetheless, most companies also use external vendors for some of their ADME PGx needs. There is some heterogeneity among the quality standards that are followed by internal PGx labs (question 18). Conformity with good clinical practices (GCP), the standards for conduct of all pharmaceutical clinical research, is uniform. However, GCP standards do not address most aspects of laboratory quality. Most pharmaceutical companies have borrowed quality standards from good laboratory practices (GLP). Although some parts of GLP (which were developed for animal research) cannot be directly applied to PGx research, certain standards such as those for laboratory instrumentation and sample tracking are useful. However, it is important to note that some aspects of GLP cannot be implemented in PGx research. Even those companies that do not profess to follow GLP in their PGx labs may use some similar standards. A minority of internal PGx labs conform to good manufacturing practices (GMP), quality standards that are necessary if a company wants to be able to use results from its DNA samples to support regulatory approval of a PGx test, such as an in vitro diagnostic for marketing in the United States. One company indicated compliance with Clinical Laboratory Improvement Act requirements, those that apply in the United States for the generation of data that will be used in nonresearch medical decisions. Use of an approved in vitro diagnostic for clinical trial use is not prevalent—nearly half of companies have used an in vitro diagnostic test in at least 1 study (question 17). Low prevalence is not surprising because there is no legal requirement to use an in vitro diagnostic test for research applications and hence no incentive to incur the additional cost.

Banking DNA samples for future PGx research beyond the initial period of the clinical trial is standard pharmaceutical industry practice (question 9). The most common practices among pharmaceutical company PGx laboratories with respect to protecting subject confidentiality are single or double coding (question 9).[1] Single coding offers the same confidentiality protection for PGx samples and data as for all other clinical trial samples and data. Double coding introduces a second unique identifier to the sample, providing an additional degree of confidentiality. A minority of companies anonymizes some or all of their PGx samples and data. This practice may limit the utility of PGx results for regulatory purposes by rendering results unauditable by regulatory agencies.

Statistical Considerations for ADME PGx Studies

Performing combined analysis on ADME PGx data from multiple clinical studies is standard pharmaceutical industry practice (question 13). This is often necessary because ADME PGx analyses generally "piggy-back" on clinical studies designed for

other purposes. In fact, few companies have taken the statistical power of ADME PGx analysis into consideration for any study with specified ADME PGx analysis plans (question 15). When individual studies do not have sufficient power for deductive testing of a PGx hypothesis, combining PGx and clinical data from several studies has been a useful approach. It is expected that most individual ADME PGx studies conducted in industry will continue to be exploratory in nature and will not be designed with "full" statistical power to answer PGx questions. Perhaps also related to the lack of formally powered ADME PGx studies, replication of results is generally considered important, particularly for results reported in a regulatory submission. However, several companies have used unreplicated results for internal decision making (question 14).

Use of ADME PGx Data Within Pharmaceutical Companies

Most companies have benefited from ADME PGx research, either in the context of regulatory submissions or for internal decision making (questions 19, 20, and 21). Furthermore, scientists from more than half of the companies surveyed have published original ADME PGx research in peer-reviewed journals (question 19). An open-ended question in the PhRMA survey asked each company to provide up to 3 peer-reviewed publications authored by their employees. The responses highlight several aspects of how companies are using PGx research and contributing to the field. Some examples are given in the following sections.

Dose Selection and Adjustments

Dose selection stratified by genotype provides an additional mechanism to address the impact of polymorphisms in DMEs and transporters. One example is warfarin, where it has been observed that polymorphisms in the CYP2C9 gene (as well as the gene encoding the pharmacological target VKORC) influence prothrombin time.[2] Genotype information in combination with nonpharmacogenetic factors (eg, age, height, body weight) provides an "algorithm" for dose selection in patients to minimize risk of excessive bleeding. This information is included in the prescription label for warfarin: "About 55% of the variability in warfarin dose could be explained by the combination of VKORC1 and CYP2C9 genotypes, age, height, body weight, interacting drugs and indication for warfarin therapy in Caucasian patients."

Another example of the use of PGx for dose selection or adjustment comes from the label for atomoxetine, a sensitive CYP2D6 substrate. Individuals lacking CYP2D6 activity have higher atomoxetine plasma concentrations after multiple doses due to lower clearance compared with extensive metabolizers.[3,4] During its development, there was concern that poor metabolizers would not tolerate higher drug concentrations and that these patients should be identified prospectively before the initiation of treatment. In early trials, patients were given doses in accordance with genotype, with poor metabolizers receiving lower doses than extensive metabolizers.[5,6] After demonstrating safety and tolerability in poor metabolizers, atomoxetine was administered without regard for genotype in later clinical studies.[7] A comparison of 1290 extensive metabolizers with 67 poor metabolizers taking at least 1.2 mg/kg/day of atomoxetine, which is the initial target dose for efficacy, showed little difference in discontinuations or reporting rates of adverse events.[7,8] Thus, due to the large therapeutic index, differential dosing based on genotype is not required for atomoxetine.

Similarly, repaglinide metabolism is affected by the CYP2C8 polymorphism, leading to reduced plasma concentrations in the CYP2C8*1/*3 genotype.[9] However, the PGx effect is not clinically meaningful as there is no fixed dosage regimen for the management of type 2 diabetes with repaglinide. Dosing is initiated at low doses, and adjustments are made based on pharmacological response (repaglinide label). The reduced PGx impact of CYP2C8 may be due to the fact that CYP3A4 also contributes to the metabolism of repaglinide.[10]

Safety

ADME genotyping activities can be useful in understanding the contribution of interindividual differences in exposure related to tolerability and/or safety of drugs. One example of the utility of this approach as part of a safety surveillance plan is that of assessing hyperbilirubinemia risk for those individuals with a compromised ability to glucuronidate bilirubin owing to the UGT1A1*28 polymorphism.[11] Most individuals homozygous for UGT1A1*28 (Gilbert's syndrome patients) display a normal phenotype but are predisposed to hyperbilirubinemia when administered inhibitors of UGT1A1.[12] This inhibitor-genotype interaction does not necessarily manifest itself as severe adverse events but does provide an example of the utility of this approach. For example, during a phase III clinical study evaluating the utility of tranilast in the prevention of restenosis following percutaneous transluminal coronary revascularization, an increase in bilirubin levels was observed in

12% of the Western population patients receiving tranilast.[13,14] To identify the possible mechanism of the hyperbilirubinemia, polymorphisms in the UGT1A1 gene were evaluated in more than 1000 patients. The results suggested that the TA repeat polymorphism in UGT1A1*28 predicted the susceptibility to the tranilast-induced hyperbilirubinemia. If tranilast had gone on to be successfully marketed, identification of subjects with Gilbert's syndrome via PGx would have been a useful test to explain changes in bilirubin levels related to this benign condition.

Another example of using ADME PGx to increase subject safety comes from work published with lamotrigine.[15] Desipramine was selected as the comparator for a pivotal study for lamotrigine in the treatment of unipolar depression. Blinded administration of desipramine, a CYP2D6 substrate, could present unacceptable safety risks to poor metabolizers (PM). Hence, CYP2D6 PM status (determined by genotype) was used as an exclusion criterion.[15] This approach successfully removed poor metabolizers prior to study enrollment and reduced the risk for adverse events in this population.

Drug-Drug Interactions

Companies have used ADME PGx to estimate or explain the drug-drug interaction potential of new chemical entities without the need for additional clinical trials. The underlying principle is that the genotype and pharmacologic inhibition of a metabolic or transport pathway should have a similar effect on a drug's pharmacokinetics. One example of this approach is with tipifarnib, a farnesyltransferase inhibitor that has been developed for use in oncology. In vitro data suggested that CYP2D6 could have a meaningful role in the disposition of tipifarnib. Understanding the potential for tipifarnib to be a victim of drug interactions mediated via CYP2D6 was important because certain CYP2D6 inhibitors such as antidepressants may be administered to cancer patients. The effect of CYP2D6 metabolizer status on tipifarnib systemic clearance was explored through genotyping subjects from 6 clinical trials. The results demonstrated that intermediate and poor metabolizers had, on average, about 95% and 96% of the systemic clearance of extensive metabolizers, indicating no need for tipifarnib dose adjustments in patients coadministered with CYP2D6 inhibitors.[16] This example highlights the principle that preclinical evidence does not always translate quantitatively into the clinical context.

Exposures to atrasentan, an endothelin antagonist that was developed for use in prostate cancer, were increased by coadministration with rifampin. This finding was unexpected, as rifampin is widely used in drug interaction studies as an inducer of metabolism. Subjects from 5 clinical trials were genotyped to assess the relationship between OATP1B1 transporter status and atrasentan clearance.[17] A monotonic decreasing trend for atrasentan clearance was observed, with "extensive transporters" having the highest clearance and "poor transporters" the lowest. Subsequent in vitro studies confirmed that atrasentan is an OATP1B1 substrate exhibiting different uptake rates between extensive and poor transport OATP1B1 alleles. This work explained the clinical trial observation and indicated that atrasentan pharmacokinetics may be influenced by OATP1B1 inhibitors, such as rifampin.

Pharmacokinetic Outliers

Companies have used ADME PGx to explain pharmacokinetic outliers in their clinical trials. For example, it was demonstrated that a healthy volunteer deficient in the conversion of losartan to its active metabolite E-3174 was a PM for CYP2C9, an enzyme expected to have a role in losartan metabolism.[18] During a CYP2D6-screened pivotal study for lamotrigine,[15] 2 individuals in the desipramine arm had plasma desipramine concentrations well above the therapeutic range. Upon further genotyping, 1 of the subjects was identified as a PM by follow-up genotyping for 2 less common CYP2D6 PM alleles. The other was identified as an intermediate metabolizer, although it is possible that this person carried a CYP2D6 PM allele for which testing was not done. For example, this individual might have carried a CYP2D6*9 allele, as was recently demonstrated as a pharmacokinetic outlier in another PM-screened study involving desipramine.[19] PGx is particularly useful for this application in the context of regulatory submissions, during which otherwise unexplained outlier data can reasonably lead to requests for additional investigations, including costly and time-consuming clinical studies focused on a specific clinical pharmacology question.

Bridging Studies

Bridging studies are necessary to link the development of a drug in 1 geographical region to the registration in another region. According to the International Conference on Harmonization (ICH) E5 guidelines, bridging studies are necessary for drug registration in Japan. The additional burden of extra costs and delayed time toward registration for this effort spurred a study to address whether a single

clinical global protocol could be put into place instead of the traditional bridging study.[20] The initial aim was to investigate genotype-phenotype correlations for the major CYPs between Caucasian and East Asian populations. The key findings of this study were that (1) based on CYP genotype and phenotype, East Asian populations could possibly be used interchangeably for drug development trials, and (2) expatriated (eg, US-based) Japanese could possibly be used for drug registration in Japan. This study represents the first step in possible replacement of bridging studies with a single clinical global protocol. The next steps include extending the above described approach to drug transporters and other ADME genes.

Negative Results of ADME PGx Associations

An underappreciated aspect of ADME PGx is that, in addition to positive results supporting the importance of a particular DME or transport protein for a drug, negative results of ADME PGx association experiments can help streamline a drug's development by identifying that certain pathways are not likely to contribute appreciably to the metabolism of the compound. A large proportion of published ADME PGx association studies have negative results. Using population pharmacokinetic analyses that incorporated CYP2D6 genotyping, the limited influence of CYP2D6 PM status on the pharmacokinetics of tipifarnib[16] and galantamine[21] was demonstrated. Furthermore, other studies have reported lack of association for genotypes in CYP3A5 and OATP1B1 with atorvastatin response.[22] Although showing positive associations for *CYP3A5* genotype with cethromycin clearance[23] and SLCO1B1 genotype with atrasentan clearance,[17] the lack of association for several ADME genes with the pharmacokinetics of these compounds was also reported. It is of particular interest that in many of these situations, positive results of the clinical ADME PGx experiments were expected based on available in vitro data. Thus, preclinical in vitro studies predicting the clearance pathways and therefore the potential PGx concerns for a compound performed prior to elucidating the clearance of the candidate in humans should be viewed as hypothesis generating and not definitive information. This sort of ADME PGx evidence points away from the need to perform additional clinical trials (eg, drug-drug interaction studies) or to use tailored dosing later in development, thus saving both time and money as a new chemical entity moves toward registration. Negative results should be interpreted with caution because the lack of association does not necessarily imply that a given enzyme does not contribute to the metabolism of the compound.

Current ADME PGx Tests

Using PGx to improve patient care is a laudable and well-publicized goal. Today, a few genetic tests are common in clinical practice. Each of these tests meets 3 important criteria: (1) addresses a medical need, (2) is clinically relevant, and (3) has adequate sensitivity and specificity. When these 3 criteria are met for a genetic test, drug labeling should include language about genotyping. If clinical data support safety and efficacy for the drug in the general patient population without genotyping, the label should inform physicians of the impact of genotype or phenotype and indicate the availability of tests as optional. In contrast, if available data support drug safety and efficacy only in a subset of patients defined by genotype or phenotype, the label should require testing via either a specific indication or contraindication. These principles are consistent with current FDA practice, as illustrated by the following examples.

Although many marketed drugs are substrates of the polymorphic enzyme CYP2D6, only thioridazine is contraindicated in poor metabolizers. This is the only example of drug labeling that contraindicates a drug based on ADME PGx: "Thioridazine is contraindicated with these drugs [CYP2D6 inhibitors] as well as in patients, comprising about 7% of the normal population, who are known to have a genetic defect leading to reduced levels of activity of P450 2D6" (http://www.fda.gov/cder/genomics/genomic_biomarkers_table.htm). When thioridazine became contraindicated in CYP2D6 PMs in 2000, 2 key aspects of its clinical pharmacology had been established. First, thioridazine concentrations are substantially higher in this population.[24] Second, a relationship between thioridazine dose and prolongation of the QT interval had been established.[25] Thioridazine (300 mg) was studied as a comparator agent for ziprasidone (FDA internal memo of June 14, 2000). In this study, thioridazine was associated with a mean Bazett-corrected QT interval (QTc) increase of 36 msec, with QTc increases of at least 30 msec in 30/31 patients and a correlation ($r^2 = 0.25$) of QTc with plasma concentration. Such electrocardiograph changes have been associated with serious ventricular arrhythmias and sudden death.

The labels for mercaptopurine drugs were updated in 2004-2005 to include a warning that TPMT PMs may be unusually sensitive to myelosuppressive effects of mercaptopurines and prone to developing rapid bone marrow suppression following the initiation of treatment. The labels also note that substantial dosage reductions may be required in TPMT PMs. Relationships between TPMT activity, mercaptopurine concentration, and risk of myelosuppression are long established.[26] Although myelosuppression can be fatal, the toxicity of mercaptopurines can be managed through monitoring and dose adjustment. This is a key difference compared with thioridazine and accounts for the difference in label wording between the drugs.

Because the adverse effects of thioridazine or mercaptopurines can be fatal, there is considerable medical need to understand which individual patients are at the highest risk. CYP2D6 and TPMT tests, respectively, meet this need because PMs are at extreme risk. For these drugs, CYP2D6 or TPMT testing also meets the standard of clinical relevance because any result of the test can aid a physician's decision related to an individual patient. The benefit-risk profiles and dosing recommendations (for mercaptopurines) are understood not only for PMs but also for intermediate metabolizers (IMs) and extensive metabolizers (EMs). Finally, clinical experience has demonstrated substantially reduced toxicity if genotype-based clinical decisions are implemented, thus demonstrating useful sensitivity and specificity for the genotype tests.

Similarly, irinotecan is a chemotherapeutic agent often used in combination therapy. Irinotecan is metabolized to its active metabolite SN-38, which is in turn inactivated by UGT1A1 and possibly other UGT enzymes. Several polymorphisms exist within the UGT1A1 gene. The best studied polymorphism is that of promoter repeats of TA dinucleotides—those individuals homozygous for 7 promoter TA repeats (carrying 2 copies of the UGT1A1*28 allele) express a 2.5-fold lower level of UGT1A1 protein than those with 6 TA repeats (carrying 2 copies of the UGT1A1*1 allele).[11] A recent laboratory study investigating genotype/phenotype correlations for promoter TA repeats concluded that the UGT1A1*28 allele contributed only 40% of the total observed variability in UGT1A1 enzyme activity. In 2004, a small clinical study showed an association of the UGT1A1*28 variant with greater risk of severe neutropenia.[27] In 2005, the FDA requested changes to the labeling of irinotecan to include recommendation for dosage adjustment based on UGT1A1 TA promoter repeat genotype information. More recently, subsequent publications[28,29] based on larger studies have weakened the original claims for influence of the UGT1A1 genotype on the risk of severe neutropenia. Correspondingly, the European regulatory agency (EMEA) has decided not to recommend dosage adjustment based on UGT1A1 genotype. Recently, the FDA has approved a clinical test for the UGT1A1*28 allele.[30] Along with TMPT, the irinotecan label includes information about genotype variants that may affect the pharmacokinetics and toxicity following drug treatment[31]; however, application of this information in clinical practice is low.

A counterexample is that CYP2C19 genotyping has not been implemented in selecting therapy for *Helicobacter pylori* eradication in peptic ulcer patients, resulting in no labeling changes being publicly considered by regulatory agencies. The response rate to 2-drug therapy of amoxicillin and a proton pump inhibitor is lowest in EMs and highest in PMs.[32,33] Response rate to 3-drug therapy (adding a second antibiotic) is high regardless of CYP2C19 metabolizer status.[34] However, this regimen is more expensive and may not be as well tolerated by all patients. A test to aid in making the decision whether to prescribe 2 or 3 drugs for initial therapy would meet an important medical need. However, CYP2C19 genotyping does not meet the other 2 criteria of clinical relevance and adequate sensitivity/specificity. Many EMs will have a good response to 2-drug therapy. The additional risks of 3-drug therapy are more in the realm of tolerability than toxicity. Hence, CYP2C19 genotype test results are unlikely to substantially influence a physician's decision between 2- and 3-drug regimens.

To generalize from the above examples, medical need and clinical utility for a PGx test based on drug disposition genotypes depend on the particular drug and indication. Relevant drug properties include the strength of the pharmacokinetic-pharmacodynamic relationship, the size of the therapeutic window, the potential need for dose titration or adjustment, the extent of interindividual efficacy variation, the severity and prevalence of adverse events, the pharmacology of metabolites in addition to the parent drug and the drug interaction potential. Aspects of the medical context are also relevant, including the potential morbidity or mortality, the frequency of patient-clinician interaction, and the availability of alternate therapies.

Factors for Improved Use of PGx in the Future

Prospective Strategies for Application of PGx

A number of strategies for the application of ADME PGx exist, including (1) strong candidate gene, (2) all candidate genes, (3) routine screening of a short panel of "polymorphic" enzymes, and (4) high-throughput screening approaches. Many companies currently use these or a combination of these approaches. The strong candidate gene approach is a focused strategy guided by preclinical drug metabolism data (proposes a threshold value of at least 30% of the clearance of the agent dependent on metabolism/transported by a given polymorphic gene) or previous information about the drug or compound series. If this criterion is met, genotyping of that gene(s) is implemented in clinical trials. The limitation of this approach is that it hinges on the translatability of preclinical data to the clinical context. Therefore, the genes that do not meet the "threshold" are not assessed, which may result in the clinical PGx analyses guided by this approach overlooking important metabolic/elimination pathways.

The "all candidate genes" approach involves genotyping any ADME gene speculated or known to contribute to the metabolism of the compound. There are no quantitative thresholds to be met in this strategy, but existing scientific evidence generally limits the number of ADME candidate genes examined.

The routine screen approach is a proactive strategy using a panel of common polymorphic ADME related genes that are responsible for the clearance of most pharmaceuticals. This strategy does not rely on preclinical or other evidence for gene selection and allows the identification of clinically relevant drug distribution pathways to be explored on a routine basis. Consequently, this strategy permits the "ruling out" or "ruling in" of specific polymorphic ADME genes as major players in the drug disposition in vivo.[35] This may also help with forecasting the variability in drug exposure in the general population because the distribution of ADME polymorphisms is known in various human populations.[35]

The high-throughput approach makes use of technologies that allow for the simultaneous analysis of a large number of ADME genes (eg, ADME single nucleotide polymorphism [SNP] chip). Although this hypothesis-free approach has the potential to identify uncommon mechanisms, its major limitations are the increased resources for analysis, large amounts of data generated, and higher rate of false positives.

The FDA *Guidance for Industry on the Pharmacogenomic Data Submissions* (March 2005) proposes genotyping strong and all candidate genes, including those genes classified as valid biomarkers.

ADME-PGx Studies, Drug Label, and Education

Per the FDA *Guidance for Industry on the Pharmacogenomic Data Submissions* (March 2005), submission of pharmacogenomic data is expected for all known or probable valid biomarkers. The amount of data and information to be submitted (ie, full vs abbreviated report) depends on whether the data are used to support scientific arguments or be included in the label. For other types of data, such as those generated on ADME genes with less knowledge about functional variants (ie, exploratory biomarkers), the FDA is encouraging the voluntary submission of these data. There are many examples of applications and drug labels containing information about the implication of a specific ADME gene related to drug efficacy or toxicity, the recommendation of testing to be performed prior to drug administration, or, more rarely, the requirement of testing to be done. As PGx information is incorporated into labels in the future, appropriate education of prescribing physicians, ethical committees, and investigators on the use and interpretation of ADME information will be needed to facilitate the uptake of genetic testing into the clinical practice.

Unification of Standards for Sample and Information Use

The pharmaceutical industry has gained considerable experience in recent years in understanding how ADME PGx can be applied to clinical development, despite a relative paucity of guidelines and global harmonization. It has become apparent that there is no one-size-fits-all paradigm for implementing DNA sample collection in a global context. Not only do requirements vary among countries, but these can also vary on state, provincial, and local levels. DNA and the information contained within are often viewed as being exceptional and therefore receive particular attention from ethics committees and regulatory bodies. Apprehensions appear to stem mainly from the heritable nature of DNA, the possible misuse and misinterpretation of genetic data, the shortage of laws and regulations regarding the misuse of these data, and the fear of stigmatization and discrimination. These concerns are certainly valid as regards genetic data related to Mendelian diseases, yet there is substantial need for education to facilitate understanding of the different spectrum of information risk associated with pharmacogenetic

data. In addition, the banking of samples for future broader use also raises valid concerns for obtaining consent that is truly "informed," although this is not unique to DNA samples. Ethics committees and subjects are generally agreeable to sample storage for future broader research provided that there are sufficient assertions that processes and standards for subject privacy protection are in place. ADME PGx samples and data are often coded identically to other clinical trial information. As well, pharmaceutical companies have often taken additional privacy procedures that limit the possibility of linking genetic data back to a subject's identity, which can be accomplished by (1) de-identification (or double coding) of samples such that a coded sample is relabeled with a unique second code and the link between the 2 codes maintained or (2) anonymization of the samples such that the link between the 2 codes of a double-coded sample is permanently deleted (see previous example for definitions used). The pharmaceutical industry, in general, appears to have developed and implemented stringent and appropriate procedures to allow for the routine collection of DNA samples for PGx applications in clinical trials.

In the past few years, the Pharmacogenetics Working Group (PWG) has made progress in standardizing nomenclature and informed consent forms used in PGx clinical trials. In general, this has led to a more consistent approach to the approval of informed consent forms and protocols for PGx sample collection by ethics committees and regulatory bodies in different countries. However, it may be years before harmonization is completed. Differences include sample importation or exportation regulation to control data generation from DNA samples or lengthy exportation/importation applications that can result in delays in sample procurement. Furthermore, limits can be placed on the type of research, location of sample storage, coding of samples, and rights to sample data. Another topic of debate within the PGx community has been whether and how individual genetic research results should be returned to study participants; a paper from the PWG has summarized the key points to be considered when making these decisions.[36]

Summary of the PhRMA PGx Survey and Impact of PGx in Drug Development

Genotyping during pharmacokinetic (PK) trials allows for the in vivo identification, confirmation, or exclusion of clearance pathways that are important in a drug's disposition. ADME PGx analyses can be used to (1) explain variability in the PK for a drug (or interacting drug) and implications of drug interactions, (2) ensure a trial is conducted in a balanced or representative population (ie, include or exclude variants of interest), (3) increase safety of patients, and (4) provide mechanistic information. ADME PGx studies will provide data that support claims on PK, dosing, ethnic variability, and safety concerns. Current efforts are ongoing to educate various bodies toward harmonization and standardization of regulations, as well as practices applicable to PGx globally. Appropriate education of prescribing physicians, ethics committees, and investigators on the use and interpretation of ADME PGx information provided in the drug label is needed to facilitate the uptake of genetic testing into clinical practice for improved dosing and minimization of safety events.

REVIEW OF CLINICALLY SIGNIFICANT POLYMORPHISMS IN DRUG-METABOLIZING ENZYMES AND TRANSPORTERS

To date, a number of DMEs and transporters have been shown to influence the disposition of drugs and metabolites. This section provides brief reviews of the key polymorphisms (defined as clinically meaningful) in the major DMEs and transporters. A summary of the clinically important enzymes and transporters is presented in Tables I through III.

Cytochrome P450 Enzymes

CYP1A2

CYP1A2 gene and function. CYP1A2 is the member of the aryl hydrocarbon receptor (AhR)–regulated CYP1 family constitutively expressed in the human liver. The human CYP1A2 gene is located on chromosome 15 in a head-to-head arrangement with CYP1A1.[37] In the general human population, the enzyme accounts for about 10% to 15% of total hepatic cytochrome P450, and this is also the estimated proportion of drugs metabolized by the enzyme.[38] Compounds particularly dependent on CYP1A2 for elimination include phenacetin, olanzapine, mexiletine, lidocaine, R-warfarin, tacrine, clozapine, dacarbazine, riluzole, pimobendan, methylxanthines (caffeine and theophylline), and the muscle relaxants tizanidine and zoxazolamine. Endogenous substrates include estrogens, retinoids, bilirubin, and melatonin. Initially, it was hypothesized that the high lethality seen in the first gene

Table I Summary of Allele Numbers and Functional Effects of CYP Polymorphisms

CYP Gene	Number of Alleles	Example Substrates	Functional Effects
1A2	35	Duloxetine, alosetron[a]	↓ induction ↓ expression
2A6	50	Nicotine, tegafur	↑ activity ↓ activity
2B6	>50	Efavirenz, cyclophosphamide	↓ activity
2C8	16	Replaginide[a]	↓ activity
2C9	35	Warfarin, phenytoin[b]	↓ activity
2C19	25	Omeprazole[a]	↑ activity ↓ activity
2D6	>100	Desipramine[a]	↑ metabolism ↓ metabolism
2E1	13	Isoniazid	↓ expression
3A4	40	Eplerenone, simvastatin[a]	Polymorphic expression
3A5	20	Tacrimolus[b]	Polymorphic expression

Modified from Ingelman-Sundberg[342] and Daly.[343] The number of alleles was determined from http://www.cypalleles.ki.se/. Clinical effects with parentheses indicate equivocal information. The Food and Drug Administration's Web page on drug interaction does not differentiate the substrates for the different CYP isoforms but instead provides a list of CYP substrates that are either sensitive substrates or have a narrow therapeutic range.
a. Sensitive substrates as defined by the Food and Drug Administration.
b. CYP substrate with a narrow therapeutic range as defined by the Food and Drug Administration (http://www.fda.gov/cder/drug/drugInteractions/tableSubstrates.htm#classSub).

Table II Summary of Allele Numbers and Functional Effects of Selected Phase II Enzyme Polymorphisms

Gene	Number of Alleles	Example Substrates	Functional Effects
TPMT	≥20	Thiopurines	↓ activity
COMT	≥2	Dobutamine, L-dopa	↓ activity
SULT1A1	>15	Acetaminophen, minoxidil	↓ activity
NAT2	>50	Isoniazid, amonoafide	↓ activity
UGT1A1	>30	Irinotecan	↓ express
UGT2B7	>30	Morphine, zidovudine	↓ activity
GSTM	≥3	Metabolites of polyaromatic hydrocarbons	↓ expression
GSTT	≥1	Halogenated hydrocarbons	↓ expression

Clinical effects with parentheses indicate equivocal information.

Table III Summary of Allele Numbers and Functional Effects of Selected Drug Transporter Polymorphisms

Gene	Number of Alleles	Example Substrates	Functional Effects
ABCB1	>100	Digoxin, fexofenadine	Unclear
ABCC2	>200	Indinavir, cisplatin, drug conjugates	↑ activity ↑ expression
ABCG2	>40	Doxorubicin, rosuvastatin	↓ expression
SLC01B1	>20	Pravastatin, rifampin	↓ affinity
SLC22A1 (OCT1)		Metformin, desipramine	↓ affinity

Clinical effects with parentheses indicate equivocal information.

deletion study in mice was due to the lack of an important function provided by the enzyme[39]; however, a later study demonstrated that CYP1A2 knockout mice have normal viability.[40] The dispensable nature of CYP1A2 has since been confirmed by the finding that approximately 15% of normal beagle dogs lack a functional CYP1A2 gene due to the presence of the C1117T nonsense mutation.[41,42] In the human population, there is considerable interindividual variation in the expression of CYP1A2, which

is largely due to induction by polycyclic aromatic hydrocarbons found in tobacco smoke, char-grilled meat, cruciferous vegetables, environmental contaminants, and drugs such as the relatively weak inducer omeprazole. However, not all of the interindividual variability in human CYP1A2 can be attributed to the environment, and a proportion of the variability appears to be heritable.[43]

CYP1A2 polymorphisms. More than 2 decades ago, it was noted that the human metabolism of theophylline in vivo is polymorphic,[44] with family and twin studies suggesting at least 2 alleles. In vitro studies also found polymorphism in the population distribution of microsomal N-hydroxylation of 2-AAF[45] and the metabolism of phenacetin.[46] Early attempts to identify the source of the polymorphism met with little success[47,48] because these attempts focused mainly on the coding regions of the gene. Recent studies have shown that the most common mutations affect the regulation of expression, rather than the protein sequence. The CYP1A2*1C allele is present at relatively high frequencies and is reported to result in reduced inducibility of CYP1A2 in Japanese smokers,[49] whereas the frequency of the CYP1A2*1K allele, another shown to reduce inducibility, shows strong ethnic variation.[50,51] Other promoter mutations resulting in reduced constitutive activity and/or inducibility are still being discovered, and haplotypes are being identified.[51] The CYP1A2*1F allele is also found at relatively high frequencies and actually results in higher inducibility (the so-called ultra-rapid phenotype). This mutation has been associated clinically with more extensive caffeine metabolism in 2 studies[52,53] but not in another of 600 subjects of various East Asian ethnicities and Caucasians[20] or another study of 350 Japanese.[54] The CYP1A2*1F allele has also been associated with fluvoxamine-inhibitable nonresponsiveness to clozapine.[55,56] Although regulatory polymorphisms appear to be quantitatively the most important for CYP1A2, mutations affecting the protein sequence have also been observed, but all have been detected at lower frequencies. More than 35 variants for CYP1A2 are listed at the Human Cytochrome P450 Allele Nomenclature Committee Web site (www.imm.ki.se/cypalleles/), and other coding SNPs are still being characterized.[57,58] Most of these changes have little apparent effect on function,[20] the confirmed exceptions being CYP1A2*6 leading to absence of protein,[59] CYP1A2*11 resulting in reduced activity,[60] and CYP1A2*8, CYP1A2*15, and CYP1A2*16 also resulting in reduced activity.[61]

Summary for CYP1A2. Mutations leading to less functional or absent protein have been described for CYP1A2, but the most common mutations are those affecting the regulation of the enzyme with most focus on CYP1A2*1C and CYP1A2*1F. The indirect nature of their effects renders the clinical manifestations of the mutations difficult to predict as they would be dependent on the relative exposure of the subjects to inducing agents.[62] The ultimate clinical relevance of the CYP1A2 genetic polymorphism is still the subject of dispute, with antipsychotic therapy receiving the most attention; some groups provide evidence of a clear effect of genotype,[55,56] some show no influence,[20] and others argue that the environmental influences dominate instead.[63,64]

CYP2B6

CYP2B6 gene and function. The CYP2B6 gene is located at chromosome 19q13 near the middle of the 400-kilobase CYP2A-CYP2B-CYP2F cluster and lies contained within the CYP2A7P1 pseudogene.[65] The role of CYP2B6 in drug metabolism has been relatively overlooked until recently. This neglect was likely due to a number of factors: the high level of aberrant CYP2B mRNA splicing that impeded obtaining full-length cDNAs, the low level of CYP2B6 protein expression in most individuals (<1% of total hepatic cytochrome P450 content), and the lack of good marker activities and selective inhibitors.[66] The situation has now completely changed, with CYP2B6 being recognized as the catalyst for the metabolism of several clinically very important drugs and potential use in gene therapy.[67]

Bupropion 4-hydroxylase has become a preferred activity for assaying the enzyme in vitro, largely supplanting S-mephenytoin N-demethylase, and has the advantage that it is also a useful clinical probe for CYP2B6 activity[68] and pharmacogenetic studies.[69] CYP2B6 is involved in the metabolism of other neuroactive agents, such as mephobarbital, methadone, tramadol, ketamine, clotiazepam, selegiline, meperidine, propofol and piclamilast, the antidiarrheal loperamide, and the antiplatelet agent prasugrel. CYP2B6 also plays an important role in the activation of a number of oncolytics through 4-hydroxylation of the oxazaphosphorine anticancer agents, cyclophosphamide, ifosfamide, and trofosfamide, and can inactivate the antimitotic agent, rhazinilam, and the antiestrogen, tamoxifen. Another anticancer agent, N,N′,N″-triethylene thiophosporamide (ThioTEPA), is a relatively selective mechanism-based inactivator of

CYP2B6 and, along with 2-phenyl-2-(1-piperidinyl)-propane (PPP), has largely replaced early use of orphenadrine as a diagnostic inhibitor.[70]

CYP2B6 polymorphisms. The expression of CYP2B6 shows wide interindividual variation. This is partly due to environmental influences as transcription of the gene can be increased in response to activation of the constitutive androstane receptor (CAR) or pregnane X receptor (PXR).[71] Genetic polymorphisms in CYP2B6 have been recognized only within the past 5 years. The human cytochrome P450 allele Web site (www.imm.ki.se/cypalleles/) currently lists more than 50 CYP2B6 alleles, about a quarter of which primarily affect the promoter. Those CYP2B6 alleles that have been characterized functionally generally lead to decreased expression and/or activity, the exceptions being CYP2B6*22, where enhanced transcription was observed,[72] and the K262R mutation, where in a study using a truncated form of the enzyme, substrate-dependent increases in activity were seen.[73] Recently, a more severe deletion, CYP2B6*29, was reported in 1 individual, and this arose through crossover with CYP2B7.[74]

Although the CYP2B6 polymorphism may affect treatment with cyclophosphamide,[75] much of the current interest in CYP2B6 pharmacogenetics stems from the finding that the enzyme is the principal catalyst of the hydroxylation of efavirenz and also plays a major role in nevirapine metabolism. Several CYP2B6 pharmacogenetic studies have observed impaired metabolism of efavirenz and nevirapine in the clinic, in some cases resulting in intoxication.[76-78] The genotype-phenotype correlation has also been demonstrated in vitro with efavirenz as the substrate.[79] Because anticipation of toxicity also leads to a tendency to underdose patients with wild-type CYP2B6, genetic testing and rational dose adjustment have been suggested[80,81] and, in at least 1 case, used successfully.[82] Population studies have also uncovered important ethnic differences in CYP2B6 allele frequencies, which may help improve the design of therapeutic regimens.[83,84] In addition to influencing AIDS therapy, CYP2B6 also plays important roles in the metabolism of artemisinin and β-arteether, antimalarial agents used in the treatment of drug-resistant forms of *Plasmodium falciparum*.[85] Thus, it is likely that there will be continued interest in CYP2B6 pharmacogenetics as advances are made in the therapy of another major human disease.

Summary for CYP2B6. Although the results from the various studies are somewhat variable, the clinical data with agents such as efavirenz and cyclophosphamide suggest that the Q172H mutation, which is part of CYP2B6*6, *7, *9, *13, *19, and *20, results in increased exposure to substrates. Therefore, analysis of this mutation in the clinical development of substrates primarily cleared by CYP2B6 should be considered.

CYP2C

CYP2C genes and function. The CYP2C18, CYP2C19, CYP2C9, and CYP2C8 genes are located adjacent to each other on chromosome 10q24. These genes share >82% amino acid identity but exhibit relatively little overlap in substrate specificity.[86,87] The CYP2C subfamily accounts for approximately 20% of the total cytochrome P450 content in the liver, of which CYP2C9 is the major contributor.[88] CYP2C9 is involved in the metabolism of a number of drugs, including diclofenac, tolbutamide, warfarin, and losartan. The 2 forms, CYP2C8 and CYP2C19, are present at lower concentrations but are active toward numerous drugs, whereas CYP2C18 is considered to be of little importance to drug metabolism.[89,90] CYP2C19 is involved in the metabolism of the S-mephenytoin and the proton pump inhibitors such as omeprazole.[91] CYP2C8 has been recognized during recent years as an important drug-metabolizing enzyme responsible in part for the metabolism of at least 5% of drugs cleared by phase I oxidations.[90] Several of the drugs metabolized by CYP2C8 are also metabolized by CYP3A4. Amodiaquine was recently described as a selective probe substrate for CYP2C8[92] but was earlier misclassified as a CYP3A4 substrate. Other drugs that are mainly metabolized by CYP2C8 include amiodarone, paclitaxel, cerivastatin, and rosiglitazone.[90] The CYP2C forms are expressed in both the liver and the gastrointestinal tract and exhibit large interindividual variations in protein expression, reflecting both transcriptional regulation by exposure to exogenous- and endogenous-inducing compounds as well as polymorphisms in the CYP2C genes.[88,92,93] The CYP2C19 phenotypes form distinct EM and PM populations, describing genotypes that express or do not express the enzyme. The various CYP2C9 and CYP2C8 alleles produce enzymes with mainly an altered activity, with very few allelic variants having been found that do not produce an active enzyme. The identified linkage between CYP2C8 and CYP2C9 alleles that produces both enzymes with decreased activity may be of importance for drugs metabolically cleared by both CYP2C forms.[94] The short distance between the

location of the CYP2C genes favors possible genetic linkages. Therefore, a better understanding and knowledge of CYP2C haplotypes may lead to insights into drug metabolism, which may have implications for clinical practice.[95,96]

CYP2C8

CYP2C8 polymorphisms. The Human Cytochrome P450 Allele Nomenclature Committee Web page (www.imm.ki.se/cypalleles/) currently indicates 16 alleles of CYP2C8. CYP2C8*2 is described for a black population with a frequency of 18% but is very rare in Caucasian subjects, whereas CYP2C8*3 has a frequency of 13% to 23% in Caucasians and 2% in blacks. The CYP2C8*4 allele has a frequency of 8% in Caucasians, and the CYP2C8*5 allele has been described in a Japanese population with an allele frequency of 0.25%.[90]

Clinically significant CYP2C8 polymorphisms. CYP2C8.2 protein has a single amino acid substitution (Ile269Phe), and CYP2C8.3 has 2 amino acid substitutions (Arg139Lys, Lys399Arg). CYP2C8.2 showed reduced paclitaxel hydroxylation activity in vitro by 15%, whereas the activity for CYP2C8.3 was below the level of detection.[97] However, the metabolism of amiodarone was not significantly different for CYP2C8.3 compared with CYP2C8.1.[98] Evidence for clinically significant polymorphism has been described for CYP2C8*3. Repaglinide AUC was unexpectedly 45% lower in CYP2C8*1/*3 than in CYP2C8*1/*1 individuals.[9] A strong correlation between the CYP2C8*3 allele and reduced clearance of R-(–)-ibuprofen has been observed.[99] The ibuprofen clearance in individuals homozygous or heterozygous for both CYP2C9*3 (described below) and CYP2C8*3 was only 7% to 27% of that in CYP2C8*1/*1 individuals. The linked gene combination CYP2C8*1/*3 and CYP2C9*1/*2 was present in 20% of the individuals and exhibited changes in most ibuprofen pharmacokinetic parameters when compared with CYP2C8*1/*1 individuals.

Summary for CYP2C8. Based on the current knowledge of the metabolic capacity, frequency, heterogeneous ethnic distribution, and clinical consequences of the CYP2C8*2 and CYP2C8*3 alleles, testing for these 2 forms could be helpful when administering drugs mainly cleared by this enzyme.

CYP2C9

CYP2C9 polymorphisms. The Human Cytochrome P450 Allele Nomenclature Committee Web page (www.imm.ki.se/cypalleles/) currently indicates more than 35 variants of CYP2C9. The CYP2C9*2 (Arg144Cys) and CYP2C9*3 (Ile359Leu) alleles are present in approximately 10% of Caucasians.[100] A genotype frequency analysis showed that 33% of the Caucasian population possess 1 or more CYP2C9*2 or *3 alleles.[101] The CYP2C9 polymorphisms exhibit large interethnic variability.[102] In the black American population, the frequencies of CYP2C9*2 and CYP2C9*3 variants are less common, showing a frequency of 0.5% to 1%. The CYP2C9*2 variant has not been described in Asian populations, but the frequency of the CYP2C9*3 variant is 1.1% in Korean, 2.2% in Japanese, and 3.3% in Chinese populations. In addition to these relatively common CYP2C9 alleles, CYP2C9*4 (Ile359Thr) was identified in a Japanese patient.[103] The CYP2C9*5 (Asp360Glu) allele has been identified in an African black population (3%), which yields a reduced activity similar to CYP2C9*3.[104] The putative PM CYP2C9*11 allele was present in 6% of an African population and may thus be an important genotype in black populations.[105] A null allele, CYP2C9*6, has been identified in 1 individual with severe phenytoin toxicity.[106] Additional CYP2C9 alleles, CYP2C9*7 through *23, have been described, most of them at low frequency in certain ethnic groups or geographical areas.[96,105,107]

The CYP2C9.2 and CYP2C9.3 proteins generally exhibit a reduced catalytic activity with increased K_M values and/or decreased V_{max} values, resulting in decreased apparent intrinsic clearance (V_{max}/K_M) for various substrates.[108] The CYP2C9.2 protein tends to have less impact on the intrinsic clearance than the CYP2C9.3 variant. The reduction in metabolism for the CYP2C9.3 variant is substrate dependent. The intrinsic clearance of CYP2C9.3 for diclofenac 4′-hydroxylation decreased 3- to 4-fold, whereas a 27-fold reduction was seen for piroxicam 5′-hydroxylation intrinsic clearance.[109] The intrinsic in vitro clearance of tolbutamide hydroxylation by CYP2C9.11 was 60% of CYP2C9.1.[105]

Clinically significant CYP2C9 polymorphisms. There are numerous studies indicating the clinical importance of CYP2C9 polymorphisms. The CYP2C9*3/*3 genotype has been shown to have the most dramatic effects on reduction of the in vivo clearance of several CYP2C9 substrates such as tolbutamide, losartan, phenytoin, glipizide, and a number of nonsteroidal anti-inflammatory drugs (NSAIDs).[102,110] A more modest reduction in metabolism has been observed in heterozygous genotypes (eg, *1/*2, *1/*3) or homozygous *2/*2 or compound heterozygous *2/*3 genotypes.[111]

There are several examples where CYP2C9 poor metabolizers experienced higher incidences of adverse drug reactions. The odds ratio for gastrointestinal bleeding incidences in patients after NSAID treatment was significantly higher in patients carrying CYP2C9*2.[112] In diabetic patients treated with the hypoglycemic drug glimepiride or glibenclamide, the odds ratio for severe hypoglycemia was significantly increased in the CYP2C9 genotypes *3/*3 and *2/*3.[113]

Treatment with warfarin, an oral anticoagulant that is a vitamin K antagonist, is complicated because of its narrow therapeutic index and complicated dose-response relationship.[114] A standard feature of its use is individualization of dosing, which partly can be explained by the polymorphic nature of its metabolism. Warfarin is a racemate, and the S-enantiomer is primarily responsible for its pharmacology and mainly metabolized by CYP2C9. The clearance of S-warfarin was found to be 3-fold lower in individuals being homozygous for CYP2C9*2 and 10-fold lower in homozygous carriers of the CYP2C9*3 allele.[115] A clear correlation between warfarin dose requirements and CYP2C9 genotype has been established, which further stresses the importance of understanding the impact of CYP2C9 polymorphism in clinical practice.[2] The results from a study of the CYP2C9 haplotype structure in European American patients stabilized on warfarin therapy indicate that information on CYP2C9*2 and CYP2C9*3 alleles was important for the dosing regime, whereas genotyping for other CYP2C9 allelic variants was not useful.[96] In addition to CYP2C9 polymorphisms, mutations in the vitamin K epoxide reductase, which is the target for vitamin K antagonists, were shown to have an important impact on warfarin response.[116,117] Genotyping of both CYP2C9*3 and 2 SNPs on the vitamin K reductase gene (VKORC1) was found to predict about 50% of the interindividual variability of the pharmacodynamics of the warfarin anticoagulant response.

Summary for CYP2C9. There is no doubt that genotyping for CYP2C9*2 and CYP2C9*3 predicts slow metabolism of drugs cleared mainly by this enzyme. In the currently available pharmacogenetic studies, the main alleles that are predictive of low CYP2C9 activity analyzed are CYP2C9*2 and CYP2C9*3. However, other CYP2C9 alleles may be important to analyze when the geographic and ethnic distribution as well as the function of these variants are better understood. In black Africans, losartan metabolism was decreased in CYP2C9*5 and *6 variants,[118] and the genotype CYP2C9 *5, *6, and *11 variants were associated with decreased phenytoin metabolism.[118]

CYP2C19

CYP2C19 polymorphisms. In most individuals, mephenytoin is stereoselectively metabolized, and the S-enantiomer is metabolized more slowly than the R-enantiomer. However, a few individuals metabolize the S-enantiomer as slowly as the R-enantiomer, and these individuals are called PMs.[119] The reason they are PMs is that they do not express CYP2C19 because of a defect or mutated CYP2C19 gene,[120-122] which is inherited as an autosomal recessive trait. More than 25 alleles for CYP2C19 (www.imm.ki.se/cypalleles/) are recognized, of which m1 (*2), m2 (*3), and m3 (*4) are the most common.[123] Per definition, in a PM, both alleles are mutated, whereas individuals with only 1 mutated allele and 1 reference allele (heterozygotes) or individuals with 2 reference alleles (homozygotes) are called EMs. The PM frequency varies between different populations, and there are more PMs among Asians (~15%) than among Caucasians and Africans/blacks (~3%).[124] The extremes have been reported in smaller ethnic groups such as the Cuna Indians in Panama (0% PMs)[125] and Vanuatuans in the South Pacific (~70% PMs).[126] Today, a wide array of drugs has been shown to be metabolized by CYP2C19.[124]

Clinically significant CYP2C19 polymorphisms. Tricyclic antidepressants, such as amitriptyline, clomipramine, imipramine, or trimipramine, are all partly metabolized by CYP2C19, and they all show higher plasma concentrations in PMs than in EMs.[124,127-133] In an article by Kirchheiner et al,[134] a dose reduction for PMs is recommended for a number of antidepressant drugs that are CYP2C19 substrates, including tricyclic antidepressants. Selective serotonin reuptake inhibitors, such as citalopram, fluoxetine, or sertraline, are also partly metabolized by CYP2C19, and accordingly, higher plasma concentrations have been reported in PMs than in EMs.[124,135-137] Furthermore, a higher frequency of adverse effects has been reported in PMs. For barbiturates, the effect varies; for example, hexobarbital demonstrates higher plasma levels and more pronounced sedation in PMs, mephobarbital seems to result in a higher frequency of adverse effects in Japanese PMs, and phenobarbital does not show consistently higher plasma levels in PMs.[124,138-140] Two drugs with a narrow therapeutic index, phenytoin and warfarin, are partly metabolized by CYP2C19 (formation of the phenytoin metabolite R-HPPH and

part of the metabolism of the less potent R-warfarin), and the major metabolizing enzyme for both is CYP2C9.[141,142] However, patients who are CYP2C19 PMs or inhibited via CYP2C19 and CYP2C9 PMs are at risk of developing adverse effects. For proguanil or chlorproguanil, which have demonstrated a clear gene dose effect in metabolism, no correlation between metabolizer status and malaria breakthrough episodes in Tanzanian patients could be demonstrated.[143] Diazepam is about 50% metabolized by CYP2C19, but because it has a wide therapeutic window, there is no concern with the 2-fold higher exposure in PMs compared with EMs.[144,145] An important finding in this context is that the degree of decrease in diazepam clearance with inhibition of CYP2C19 correlates with the baseline clearance, so patients with the highest exposure initially will get the least increase subsequent to CYP2C19 inhibition.[146]

For proton pump inhibitors, a clear CYP2C19 gene dose effect has been demonstrated in pharmacokinetics, but the relevance for the clinical situation seems to be dependent on the location of the patient on the dose-response curve. For example, in small studies where 20 mg omeprazole, 10 mg rabeprazole, or 30 mg lansoprazole has been administered, a clear gene dose effect in clinical efficacy was demonstrated.[32,33,147] In the study with lansoprazole, it could even be demonstrated that patients with the most severe esophagitis showed least improvement if they were homozygous extensive metabolizers, which thus would place them lowest on the dose-response curve.[147] However, in a database including patients after 40 mg omeprazole, which places the patients high up on the dose-response curve, no such effect was seen.[148]

As indicated earlier, more than 25 CYP2C19 alleles are recognized by the nomenclature committee. Although it seems most appropriate to include only the *2 allele in the genotyping screen performed to determine metabolizer status in Caucasians, *2 and *3 are required for other ethnic groups.[123] In doing so, more than 90% of PMs among Caucasians and among Africans/blacks would be detected and almost 100% among Asians.[149,150] All other alleles are very rare, and only the *4 allele (<0.5%) has been detected more than once; therefore, the number of alleles to be included in genotyping is often decided on a cost-benefit basis.

For drugs mainly metabolized by CYP2C19, bridging between different ethnic groups would be appropriate as all compounds so far identified as CYP2C19 substrates appear to be metabolized equally poorly in all PMs, irrespective of defective alleles or ethnic origin.[151] An investigation of mean CYP2C19 activities among Chinese, Korean, Japanese, and Caucasian ethnicities (n = 100 subjects per group) demonstrated similar CYP2C19 phenotypes among the Asian populations, whereas mean CYP2C19 activities in Caucasians were higher, driven by a relatively low frequency of CYP2C9*2 and CYP2C9*3 genotypes.[20] By comparison, that is not the case for poor CYP2C9 metabolizers (see the CYP2C9 section). The only factor that seems to determine the difference in exposure between EMs and PMs for CYP2C19 substrates is the fraction metabolized by CYP2C19.[152] Very recently, a common promoter variant (*17) was identified with a frequency of 15% to 20% in Caucasians and 5% in Asians, showing an enhanced metabolic ratio.[153] Although the CYP2C19*17 allele had no impact on the efficacy of *H. pylori* eradication in peptic ulcer patients treated with pantoprazole,[154] the homozygous genotype was associated with lower serum concentration of escitalopram[155] and identified breast cancer patients likely to benefit from tamoxifen therapy.[156]

Summary for CYP2C19. For CYP2C19, more than 25 different mutated alleles have been detected, most of them defective (null). Reliable in vitro/in vivo correlations and genotype/phenotype correlations exist, but phenotyping shows a substantial overlap between heterozygous and homozygous EMs. Bridging between ethnic groups is appropriate. Genotyping should include minimally *2 for Caucasians and also *3 for other ethnic groups; *4 may be also considered for Caucasians, but the clinical impact of the high-activity *17 allele remains to be fully established.

CYP2D6

CYP2D6 gene and function. Of all of the CYPs showing genetic variation, CYP2D6 is perhaps the most well studied.[157-159] The CYP2D6 gene is located at 22q13.1 adjacent to the CYP2D7P and CYP2D8P pseudogenes,[160] and the protein is thought to be responsible for the metabolism of up to 20% of prescribed drugs. Although some companies have previously taken active steps to avoid developing drugs that are mainly cleared by CYP2D6, some classes of compounds are particularly associated with the enzyme and include β-blockers, antidepressants, antipsychotics, and antiemetics.[159] Quinidine is an effective selective inhibitor in vitro and allows "phenocopying" studies in the clinic where subjects who are extensive metabolizers can be rendered temporary phenotypic poor metabolizers.[161]

CYP2D6 polymorphisms. A wealth of information on CYP2D6 variants (currently more than 100 variants listed at the human allele Web page) has been accumulated, and these include a number of nonfunctional alleles (due to deletion, insertion, splicing defects, frame shifts, or premature stop codons), less functional unstable forms (eg, CYP2D6*10), and gene amplification leading to ultra-rapid metabolism.[162] As most of the common alleles have been identified, much of the recent interest lies in cataloguing ethnic differences in their frequencies,[159,163,164] but studies on the basis for the intermediate metabolizer phenotype are still ongoing.[165]

Prospective genotyping for CYP2D6 is finding favor because of the strong predictive relationship between genotype and phenotype. Genotyping may be of use in increasing the safety in the design of some clinical trials,[15] but the more general hope is that testing will improve the safety and efficacy of CYP2D6 substrates in the clinic and allow for development of CYP2D6 substrates. New observations of wider intersubject variability within genotype for larger groups (n = 100) of subjects than previously observed for smaller groups provide additional challenges in the prediction of the CYP2D6 phenotype from genotype with regard to poor, intermediate, extensive, and ultra-rapid metabolizer status.[20] However, although poor, extensive, and ultra-rapid metabolizers could all profit from this approach, most of the benefit thus far has been derived by the last group.[166] This may reflect the prevalence of high therapeutic indices among CYP2D6 substrates, so relative overdosing of poor metabolizers has less of an impact than relative underdosing of ultra-rapid metabolizers. For example, a recent prospective study found only an indication of increased frequency of cold extremities in poor and intermediate metabolizers treated with metoprolol,[167] with no evidence for increased cardiovascular, central nervous system, or sexual side effects. There are some CYP2D6 substrates with lower safety margins, such as perhexiline, where prospective genotyping has been performed to protect poor metabolizers from potential hepatotoxicity and/or neuropathy.[168] In addition, a retrospective study indicated that dosage adjustments and switching of medications in the psychiatric population were associated with the CYP2D6 genotype.[169]

Summary for CYP2D6. As already indicated, there have been a large number of alleles identified for CYP2D6, and the frequency of these alleles varies significantly among the populations examined. Furthermore, CYP2D6 alleles have been identified that result in poor metabolizer, reduced or intermediate metabolizer, and ultra-rapid metabolizer phenotypes. The majority of poor metabolizers can be defined by genotyping for CYP2D6*3, *4, *5, and *6 in essentially all population groups. The intermediate or reduced activity alleles vary somewhat by population and can be identified as CYP2D6*10 in Asians, CYP2D6*17 in blacks, and CYP2D6*41 in Caucasians. Currently, the ultra-rapid metabolizers can be defined as those containing multiple copies of CYP2D6*1, *2, or *35, but other duplications leading to increased metabolism may also exist.[170]

CYP3A

CYP3A genes and function. The human CYP3A locus, located on chromosome band 7q21, is composed of 4 functional genes: CYP3A4, CYP3A5, CYP3A7, and CYP3A43.[171] These forms play a very prominent role in the metabolic clearance of drugs, other xenobiotics, and endogenous compounds.[172,173] Estimates suggest that the CYP3A forms participate in the metabolism of 40% to 60% of drugs.[174] The CYP3A forms have somewhat different catalytic activities and are expressed to different degrees in various organs.[175,176] CYP3A4 is the major form expressed in the adult liver and small intestine.[175] In addition to high constitutive levels compared with the other CYPs in these tissues, CYP3A4 levels can be further increased by inducing agents. Thus, the levels of CYP3A4 in the liver and intestine may vary tremendously between individuals. CYP3A5 is polymorphically expressed (see below) with significant levels of the protein detected in about 20% and 50% of livers from Caucasians and African Americans, respectively.[177,178] CYP3A5 expression has also been detected in the kidney, intestine, and other organs.[175] CYP3A7 is generally regarded as the major cytochrome P450 expressed in fetal liver,[179] although it has also been detected at low levels in adult liver and other tissues.[180] The recently identified form, CYP3A43, remains relatively poorly characterized but appears to be expressed primarily in extrahepatic tissues.[181] Studies examining the catalytic activities of CYP3A4, CYP3A5, and CYP3A7 suggest that all 3 posses a wide substrate specificity, with CYP3A4 metabolizing the widest array of substrates usually at much higher rates.[176]

The human exposure to drugs that are cleared by metabolism via CYP3A forms is often quite variable. The variability is in part the result of the independent regulation of expression of the 2 major adult

forms, CYP3A4 and CYP3A5, in the liver, intestine, and various other extrahepatic organs.[171,175] Furthermore, exposure to endogenous and exogenous compounds that are inhibitors or inducers of these enzymes adds to the variable exposure of patients to CYP3A-metabolized agents.[172] As an example of this wide variability, in a study in which the exposure of a probe CYP3A substrate was determined in subjects first treated with a potent CYP3A inhibitor and then an inducer, the exposure to this substrate was shown to vary more than 400-fold.[182] On this background, the influence of genetics on the interindividual variability of CYP3A activity has been estimated to be 60% to 90%.[183] Despite this estimate of substantial genetic influence, clinical studies to date indicate that the genetic polymorphisms described below do not often contribute significantly to the variability of metabolic clearance by CYP3A enzymes.

Clinically significant CYP3A4 polymorphisms. The Human Cytochrome P450 Allele Nomenclature Committee Web page (www.imm.ki.se/cypalleles/) indicates at least 40 CYP3A4 variants. However, several recent reviews of numerous studies examining the potential clinical significance of most of these polymorphisms indicate that because of their low frequency, even the coding region variants have not consistently been associated with altered in vivo metabolic clearance of CYP3A substrates.[163,171,184-187] This lack of consistent association of the variant to pharmacokinetic effect also applies to the widely studied CYP3A4*1B allele, which is a single-base substitution in the 5′-regulatory region of CYP3A4, referred to as the putative nifedipine response element.[171] Therefore, the current state of knowledge of the in vivo effects of the variants of CYP3A4 indicates that genomic testing would bring little added value to the development of new drugs that are substrates of CYP3A4.

Clinically significant CYP3A5 polymorphisms. CYP3A5 has been known to be polymorphically expressed from its initial discovery.[177] The major underlying defects that result in this polymorphic expression have only recently been determined.[178] Currently, there are more than 20 CYP3A5 alleles recognized by the nomenclature committee, and for several of these, there are in vitro and in vivo studies demonstrating a decrease or lack of expression of CYP3A5 relative to the reference allele. The major defective variants, CYP3A5*3 and CYP3A5*6, result in aberrant splicing of most transcripts,[178] whereas for CYP3A5*7, the single-nucleotide change results in a frame shift and premature termination of the protein.[188] A relatively large body of studies has examined the contribution of these major variant CYP3A5 alleles to the variable clearance of CYP3A-metabolized drugs.[171,186,189] Interestingly, there appears to be an emerging consensus that the genotype of CYP3A5 may affect the exposure of patients to the immunosuppressant tacrolimus.[189,190] In addition, Katz et al[23] demonstrated that the exposure of ABT-773 was lower in CYP3A5-positive versus CYP3A5-negative subjects. However, an influence of the CYP3A5 genotype on the clearance of several other known CYP3A substrates has not been clearly established.[186,189] Not withstanding these later observations, it seems prudent to genotype for the major CYP3A5 variants (CYP3A5*3, CYP3A5*5, CYP3A5*6, and CYP3A5*7) because it is likely the relative catalytic efficiencies of CYP3A5 versus CYP3A4 for a given substrate will dictate whether the CYP3A5 genotype will be associated with altered clearance of the drug.

Clinically significant CYP3A7 polymorphisms. Only a few alleles (n = 7) for CYP3A7 are recognized by the nomenclature committee. One of the variants appears to be of concern for substrates that are preferentially metabolized by CYP3A7, such as dehydroepiandrosterone sulfate.[180] This variant, CYP3A7*1C, contains 60-base pairs of the promoter of CYP3A4, which appears to result in CYP3A7 expression in adults.[178] Although few clinical studies have been performed, adults with the CYP3A7*1C allele have approximately 50% lower serum levels of dehydroepiandrosterone sulfate compared to those with the reference allele.[180] Thus, genotyping for the CYP3A7*1C allele appears to be warranted should a compound be characterized as preferentially metabolized by CYP3A7.

Clinically significant CYP3A43 polymorphisms. Only 5 variants of the CYP3A43 allele are recognized by the nomenclature committee. Little is known about the metabolic capabilities of the reference allele, and even less has been done with the variants.[171] Therefore, genotyping for the variants of CYP3A43 is not warranted at this time.

Summary for CYP3A. Variants of CYP3A5 and potentially CYP3A7 have been identified that affect the metabolic clearance of only a very few of the numerous compounds known to be biotransformed by members of the CYP3A subfamily. These few examples appear to be inconsistent with the estimate that genetics are associated with 60% to 80% of the variability of the clearance of these drugs. The current data, in fact, support the conclusion that

alteration of total CYP3A activity by endogenous and exogenous compounds that are inhibitors or inducers of the various CYP3A forms has a greater influence on the variability of exposure to CYP3A substrates than do genetic polymorphisms.

Other CYPs and the Flavin-Containing Monooxygenases

For 2 additional CYPs, CYP2A6 and CYP2E1, there is substantial evidence demonstrating clinically important genetic variants. There are more than 50 CYP2A6 variants listed on the nomenclature Web site. Of these, only CYP2A6*1 and *8 demonstrate full activity, with CYP2A6*1x2 yielding ultra-rapid metabolism. In addition, there are several null activity alleles, including CYP2A6*2, *4, and *5. Finally, several alleles have been shown to have partially reduced activity relative to the reference form, including CYP2A6*6, *7, *9, and *12.[191] There is significant clinical evidence suggesting that the smoking habits (nicotine intake) of individuals are related to their metabolism of nicotine by the various alleles of CYP2A6.

There has also been a great deal of interest in the variants of CYP2E1, for which the nomenclature Web site indicates that 13 alleles have been identified. Individuals who are homozygous for the reference allele, CYP2E1*1A, have been shown to have a greater risk of developing isoniazid-induced liver dysfunction relative to individuals with lower expression of CYP2E1 as the result of inheriting the promoter variant CYP2E1*1B.[192] These clinical results are consistent with homozygous individuals expressing a higher level of CYP2E1, resulting in greater formation of the hepatotoxic metabolite of isoniazid.

There are 5 human flavin-containing monooxygenase (FMO1-5) genes and 1 pseudogene.[193,194] Of these, FMO3 appears to be the predominant form in human drug metabolism. Decreased activity has been noted for polymorphisms of FMO3 at E158K and E308G, including clinical studies indicating greater efficacy of sulindac for the treatment of familial adenomatous polyposis in patients with these 2 mutations.[195]

PHARMACOGENETICS OF THE PHASE II/CONJUGATING ENZYMES

Enzymes that catalyze the chemical conjugation of functional groups or entire molecules to substrates will also be discussed here. Such enzymes include the UDP-glucuronosyltransferases (UGTs), methyltransferases (MTs), sulfotransferases (SULTs), glutathione-S-transferases (GSTs), and the N-acetyltransferases (NATs). These groups comprise superfamilies of enzymes with several family members.

Methyltransferases

There are at least 145 MT genes identified to date (http://www.chem.qmul.ac.uk/iubmb/enzyme/EC2/1/1/), and it is estimated that several hundred are encoded in the human genome. MTs are predominantly active as monomers and use the endogenous cosubstrate S-adenosylmethionine (AdoMet) as the methyl donor. Methylation often represents a competing metabolic pathway for sulfation, glucuronidation, and/or glutathionylation.

Thiopurine S-Methyltransferase (TPMT)

TPMT gene and function. TPMT is a cytosolic monomeric protein that catalyzes the methylation of thiopurine drugs such as 6-mercaptopurine, azathioprine, and 6-thioguanine.[196,197] Thiopurines are cytotoxic agents with a narrow therapeutic index and for which methylation represents a detoxifying reaction. The human TPMT gene is localized to chromosome 6p22.3, is approximately 34 kb in length, and comprises 10 exons, 8 of which encode the protein.[198,199] There is also a processed pseudogene on chromosome 18, which is an important consideration when employing polymerase chain reaction (PCR)–based genotyping methods.[200] TPMT is widely expressed in human tissues, predominantly in human kidney, red blood cells (RBCs), liver, and gut.[200,201] TPMT is one of the most striking examples of clinically relevant pharmacogenetic variation. Indeed, this example represents the first for which an FDA labeling change has occurred such that genotyping for TPMT is recommended prior to dosing 6-mercaptopurine.

TPMT polymorphisms. The TPMT polymorphism was first recognized in biochemical pharmacogenetic studies in which variation in the level of RBC TPMT activity was found to be substantial and largely inherited.[202] There are several single-nucleotide polymorphisms within the open reading frame of the TPMT gene that alter the encoded amino acid sequence or result in a truncated protein, such as the TPMT*3 allele, the most frequent low-activity allele. Ethnic differences in the frequency of TPMT alleles have been observed. The TPMT*3A

allele is the most frequent variant allele found in Caucasians, whereas the TPMT*3C allele predominates in Asian, African, and African American populations.[196] Other inactive alleles *4 to *23 have been described as only single occurrences.[203,204] In addition, a number of variable number tandem repeats (VNTRs) in which the number of 17- or 18-bp repeat elements varies from 3 to 9 have been characterized.[205,206] Those alleles are designated V*3 through V*9, and the *V5 allele (5 tandem repeats) is in linkage disequilibrium with the TPMT*3A allele.[206]

One in every 200 Caucasian individuals carries the autosomal recessive trait of low RBC TPMT activity, and approximately 10% exhibit intermediate activity.[203,207] The TPMT*2, *3, *4, *5, *6, *7, and *8 alleles were associated with either low RBC TPMT activity when homozygously (or compound heterozygously) expressed in human subjects or intermediate activity when heterozygously expressed with the TPMT*1 allele.[198,208-213] Furthermore, mechanistic studies of recombinant TMPT*2, *3A, *3B, and *3C variants, as well as the *4 allele, have confirmed in cell culture systems the association of these alleles with low activity, low level of protein, and/or low level of mRNA expression.[198,209,214] The VNTR polymorphism (including the sum of repeats from both alleles) appears to be inversely related to TPMT activity.[205,206] It should be noted that the variation in TPMT activity associated with the VNTR is small compared with that attributed to the SNPs. The VNTR length may partially account for variation in activity within a given TPMT "subgroup" (ie, low, intermediate, or high activity), but its clinical significance remains to be established. An early clinical phenotype associated with TPMT pharmacogenetics was profound hematotoxicity in patients with very low TPMT activity at standard doses of azathioprine and 6-mercaptopurine.[202,215-217] Subsequently, several additional serious adverse drug reactions have been associated with TPMT deficiency,[196,218] whereas thiopurine dose reduction to 10% to 15% of the standard dose was well tolerated from TPMT-deficient patients.[219,220]

Furthermore, individuals who are intermediate in RBC TPMT activity may also be at increased risk of thiopurine-associated toxicities.[221] Notably, an in vitro interaction between the TPMT intermediate phenotype and use of aminosalicylate drugs has also been recognized. Particularly sulfasalazine is a potent inhibitor of TPMT, and individuals who exhibit intermediate RBC TPMT activity in the absence of drug therapy may convert to a low-activity phenotype when taking sulfasalazine-derived drugs, although the mechanism of the interaction is not fully understood.[218] Such drugs are often coprescribed with azathioprine and thiopurine, and increased active metabolites and toxicity have been observed in these circumstances.[222]

Summary for TPMT. The current body of knowledge has led to routine pharmacogenetic screening and thiopurine dosing adjustments for TPMT-deficient individuals in some practices.[201,217,219,223-225] It is recommended that routine phenotyping or genotyping for TPMT become a standard medical practice prior to dosing thiopurine drugs. In the development of new compounds metabolized largely via TPMT, it will be important to stratify drug disposition and response data by genotype or phenotype.

Catechol O-Methyltransferase (COMT)

COMT gene and function. COMT catalyzes the O-methylation of endogenous and exogenous catechols, including drugs or drug metabolites such as catechol estrogens, dobutamine, isoprenaline, L-dopa, methyldopa, and some flavonoids. A single gene locus on human chromosome 22q11.2 gives rise to the expression of 2 forms of COMT, 1 cytosolic (S-COMT) and the other membrane bound (MB-COMT).[226] Both S-COMT and MB-COMT are active as monomers, but the membrane-bound protein contains an additional 50 amino acids at the N-terminus. Biochemical pharmacogenetic studies established that the S-COMT activity was subject to significant interindividual variation and that this variation was largely inherited.[227-231] Those studies also established that the frequencies for the low-activity and high-activity alleles were approximately equal.

COMT polymorphisms. A single G to A transition at codon 108 (158 in the MB-COMT) results in a valine to methionine amino acid change. The low-activity allele encodes methionine at codon 108/158, and the high-activity allele encodes valine.[232] The Val108 allele was designated COMT*1 and the Met108 allele COMT*2. However, because these appear to be the only 2 functionally distinct alleles, they are often referred to as the high-activity (COMT-H, $COMT^{H}$) and low-activity (COMT-L, $COMT^{L}$) alleles, respectively. Allele frequencies are approximately equal (0.5) in Caucasians, whereas frequency of the high-activity allele (Val108) is 0.7 to 0.8 in Asians and African Americans.[233-235]

Studies of recombinant Val108 and Met108 COMT variants confirmed that the Met108 variant was more thermolabile and suggested that the 2 variants

did not differ in K_M or V_{max} toward dopamine (3- and 4-O-methylation) or AdoMet.[236] Dawling et al[237] observed no differences in the kinetics of the 2 allozymes toward catechol estrogen substrates. It is now recognized that the mechanism by which the Met108 COMT variant is associated with low activity is via low steady-state level of cellular COMT protein rather than a change in the intrinsic enzymology of the protein.[237,238] The association between COMT genotype and response to various psychiatric medications is an active area of research. For example, the Val108 allele has been shown to correlate with increased incidence of tardive dyskinesia following the use of antipsychotic medications in North Indian schizophrenics.[239] Of potential pharmacogenetic interest is a report of an association of the Met108 allele with an improved response of narcolepsy patients to the stimulant modafinil.[240]

Summary for COMT. Although the COMT polymorphism is clearly functionally significant, the influence of this polymorphism on clinical response of any specific drug has not been established to an extent that warrants specific recommendation with regard to dosing. However, in the development of new drugs known to be metabolized largely via COMT, it will be important to generate data addressing drug disposition and clinical response stratified for COMT*1 and COMT*2 genotypes.

Sulfotransferases (SULTs)

Sulfate conjugation reactions are catalyzed by a superfamily of cytosolic enzymes known as the sulfotransferases.[241,242] SULTs catalyze the sulfate conjugation of a wide variety of drugs, including natural and synthetic steroid hormones, thyroid hormones, isoflavones, 4-hydroxytamoxifen, acetaminophen, and minoxidil. They use the endogenous cosubstrate 3′-phosphoadenosine 5′-phosphosulfate as the sulfate donor.[241,242]

At present, at least 12 distinct human SULT isoforms, representing 3 gene families, have been identified and functionally characterized.[241-248] Functionally significant pharmacogenetic variation has been identified in several SULT genes.[242,249,250] This section focuses on genetic variation in the human SULT1A1 gene as this enzyme is most relevant to the metabolism of small-molecule drugs.

Phenol Sulfotransferase (SULT1A1)

SULT1A1 gene and function. SULT1A1 has been studied most often within the context of conjugation of phenolic drugs such as acetaminophen, minoxidil, 4-hydroxytamoxifen, and 17α-ethinylestradiol. Biochemical pharmacogenetic characterization resulted in the detection of large individual variation in SULT1A1 activity (20-fold variation) in human tissues and established that this variation had a genetic basis.[251-254] Notably, 3 major alleles appeared to account for the genetic variation.

SULT1A1 polymorphisms. As predicted by biochemical pharmacogenetic studies and segregation analysis, 3 functionally distinct SULT1A1 variants were identified. A total of 15 human SULT1A1 alleles have been identified and are defined by different permutations of 24 common SNPs.[254,255] However, these 15 alleles encode only 3 common SULT1A1 forms (SULT1A1*1, *2, and *3). The SULT1A1*2 allele has been associated with low sulfation activity. SULT1A1*2 is common in Caucasians, African Americans, Africans (frequency = 0.27-0.33), and, to a lesser degree, Chinese (frequency = 0.04).[254-257] The frequency of SULT1A1*2 was found to be 17% in a Japanese population.[258] The SULT1A1*3 allele is not common in Caucasians (frequency = 0.01). SULT1A1*3 was not detected in a population of 143 Japanese.[258] However, the SULT1A1*3 allele was frequent in an African American population (frequency = 0.23), suggesting that the functional significance of SULT1A1*3 might be pursued in this population.[257]

It appears that the SULT1A1*2 phenotype is attributable to a combination of altered intrinsic kinetics as well as low intracellular stability. The SULT1A1*3 variant is known to exhibit a higher affinity for the cosubstrate than the SULT1A1*1 or *2 variants[255] and appears to exhibit kinetics intermediate to those measured for the SULT1A1*1 or *2 variant.[259]

The clinical significance of genetic variation in SULT1A1 is less well understood than the biochemical significance. Because SULT1A1 inactivates the active metabolites of tamoxifen, 4-hydroxytamoxifen, and endoxifen, investigators have hypothesized that genetic variation in SULT1A1 might contribute to altered efficacy of tamoxifen. Nagar et al[259] have shown that cells expressing SULT1A1*2 exhibit a greater antiestrogenic response (slower growth) than cells expressing SULT1A1*1. However, Nowell et al[260] have shown that among breast cancer patients treated with tamoxifen, those homozygous for SULT1A1*1 have a significantly increased survival rate than those homozygous for SULT1A1*2, contrary to the a priori hypothesis. Furthermore, another clinical study of tamoxifen pharmacogenetics has suggested an nonsignificant association between

SULT1A1 genotype and mean plasma concentrations of 4-hydroxytamoxifen or endoxifen among women treated with tamoxifen.[261] In that study, a trend toward higher concentrations in women carrying the SULT1A1*2/*2 genotype was observed.

Summary for SULT1A1. To date, there is no compelling evidence to support a strong role of SULT1A1 pharmacogenetics in specific drug response; therefore, no specific pharmacogenetic screening with SULT1A1 prior to initiating drug therapy is recommended. Novel drugs in development that are metabolized significantly by SULT1A1 should be developed with an understanding of the contributions of SULT1A1*1, *2, and *3 to drug disposition and response.

N-Acetyltransferases

The N-acetyltransferases catalyze the acetylation of aromatic amines, heterocyclic amines, and hydrazine molecules. These enzymes have in common the use of acetylcoenzyme A as the acetyl donor. There are 2 human NAT proteins, NAT1 and NAT2. This discussion focuses on NAT2 because this gene has been shown to have pharmacologically relevant polymorphism.

N-acetyltransferases 2

NAT2 gene and function. *NAT2* is localized to human chromosome 8p22.[262-264] The NAT2 gene is intronless and contains an 870-bp open reading frame encoded within a single exon. The first indication of genetic polymorphism in NATs was the observation of a "slow acetylator" phenotype associated with isoniazid disposition.[265] This phenotype has now been attributed to several genetic polymorphisms within the human NAT2 gene.

NAT2 polymorphisms. More than 50 human NAT2 alleles have been identified to date, but only a few seem to confer functional differences to the enzyme (http://louisville.edu/medschool/pharmacology/Human.NAT2.pdf). The NAT2*14 (Arg64Gln) and NAT2*17 (Gln145Pro) variants seem to account for the majority of the "slow acetylator" phenotype in humans, with less frequent contribution by NAT2*5 (Ile114Thr), *6 (Arg179Gln), *7 (Leu268Arg), *10 (Glu167Leu), *18 (Leu282Thr), and *19 (Arg64Trp).[266] Individuals carrying 2 "slow acetylator" alleles exhibit the phenotype of a slow acetylator, whereas those heterozygous for only 1 allele exhibit the rapid acetylator phenotype.

Amonafide is an arylamine with DNA intercalating and topoisomerase II inhibition activities. Amonafide is N-acetylated to N-acetyl-amonafide, an active metabolite that contributes to the toxicity of this chemotherapeutic agent. High interindividual variability in the pharmacokinetics of amonafide was observed in phase I studies.[267] Myelosuppression has been shown to be greater in patients who are rapid acetylators than in those who are slow acetylators.[266,267] Based on these data, subsequent dosing with amonafide was recommended with regard to the acetylator phenotype such that individuals with a slow acetylator phenotype were dosed higher than those with a rapid acetylator phenotype. Amonafide, however, is no longer used clinically in part due to its variable kinetics and toxicity. Many investigators view this case as an example of the importance of understanding pharmacogenetic contributions to the kinetics and dynamics of drugs.

Summary for NAT2. As amonafide is no longer in clinical use, there are currently no marketed drugs for which specific acetylator pharmacogenetic screening is advised. However, novel drugs that are acetylated should be developed with an understanding of the contribution of the acetylator phenotype or genotype (NAT2*5, *6, *7, *10, *14, *17, *18, *19) to the disposition and response of those drugs.

UDP-Glucuronosyltransferases

Glucuronidation is a major pathway in the metabolic transformation of numerous drugs and xenobiotics. These reactions are catalyzed by a superfamily of enzymes called UDP-glucuronosyltransferases. In humans, 2 UGT families have been described, UGT1 and UGT2. These families contain at least 24 functional genes. Wide interindividual variation exists in the glucuronidation rates of many drugs and steroids; however, the molecular mechanisms responsible for these variations have been poorly characterized. Functional polymorphisms have been described for UGT1A1, UGT1A6, UGT1A7, UGT2B4, UGT2B7, and UGT2B15 (http://som.flinders.edu.au/FUSA/ClinPharm/UGT/allele_table.html). To date, variation in drug metabolism due to altered UGT activity through polymorphisms has been demonstrated with UGT1A1 and UGT2B7.

UGT1A1

UGT1A1 gene and function. The UGT1A locus is localized to chromosome 2q37 and spans over 500 kb. That locus encodes 9 biologically active gene products (UGT1A1, 1A3-1A10) and 4 pseudogenes

(UGT1A2p, 1A11p-1A13p) via alternative transcriptional initiation of 13 unique first exons spliced to common exons 2 to 5. However, pharmacogenetic variability in UGT1A1 activity has attracted the most attention due to its involvement in 2 disease states and in drug-related toxicity.

UGT1A1 polymorphisms. Currently, more than 30 allelic UGT1A1 variants have been described.[268,269] The best studied are the UGT1A1 promoter polymorphisms in the context of irinotecan disposition. This polymorphism is defined by a variable-length "TA" tandem repeat in the regulatory TATA box of the UGT1A1 gene promoter.[270] The reference form UGT1A1*1 has 6 TA repeats, whereas a common low-activity UGT1A1*28 variant has 7 TA repeats. Other polymorphic variants with 5 to 8 TA repeats have also been reported, and an inverse correlation between the number of TA repeats and bilirubin-glucuronidating activity has been reported.[271] The UGT1A1*1 allele has frequencies ranging from 0.60 to 0.62 in Caucasians, 0.46 to 0.52 in African Americans, and 0.84 in Asians. The UGT1A1*28 variant has allele frequencies ranging from 0.38 to 0.40 in Caucasians, 0.38 to 0.43 in African Americans, and 0.16 in Asians. Additional UGT1A1 polymorphisms resulting in 5 (UGT1A1*33) and 8 (UGT1A1*34) TA repeats are found only in African Americans, with frequencies ranging from 0.07 to 0.09 and 0.02 to 0.035, respectively.

Clinical syndromes associated with UGT1A1 variation are well documented. Unconjugated hyperbilirubinemias such as Gilbert's and Crigler-Najjar syndromes have been associated with UGT1A1 polymorphisms, with the UGT1A1*28 variant strongly correlated with the mild and usually asymptomatic Gilbert's disease.[272] In addition to impaired bilirubin glucuronidation, those subjects have also been reported to exhibit a decrease in clearance of acetaminophen to its glucuronide conjugate.[273]

Among the best-known pharmacogenetic examples associated with UGT1A1 involve its role in irinotecan metabolism. Irinotecan undergoes extensive metabolism that results in a complex disposition profile. Much work has recently focused on the glucuronidation of SN-38, the active metabolite of irinotecan. Although widely used in the treatment of several cancers, irinotecan may cause unexpectedly severe and occasionally fatal (only with febrile neutropenia) toxicity of myelosuppression or diarrhea. Initial studies had suggested that patients with at least 1 copy of the (low-activity) UGT1A1*28 allele glucuronidated SN-38 to a lesser extent than those with the homozygous *1/*1 genotype.[274] Subsequent studies have reported corroborative as well as contradictory results. In a study of 65 adults with gastrointestinal cancer who were treated with single-agent irinotecan at a 350-mg/m^2 dose, the UGT1A1 genotype was evaluated for association with the plasma SN-38G (glucuronide)/SN-38 AUC ratio.[275] Changes in this AUC ratio did not significantly associate with the UGT1A1 genotype. In contrast to these results, a separate study with 94 Caucasian patients with solid malignant tumors and on irinotecan monotherapy observed a significant association between the UGT1A1*28/*28 genotype and SN38G/SN-38 AUC ratio.[276] A third study with 88 Japanese cancer patients also found significant association between the UGT1A1*28/*28 genotype and decreased SN-38G/SN-38 AUC ratio.[277] In another study, carriers of the UGT1A1*28 allele showed significantly reduced SN-38 glucuronidation rates. Of those subjects, only UGT1A1*28 heterozygotes or homozygotes exhibited increased irinotecan toxicity. Finally, in a study of 66 metastatic colorectal cancer patients receiving capecitabine + irinotecan therapy, UGT1A1 genotype was found to have no association with either tumor response or the dose-limiting toxicity, diarrhea.[278] These studies suggest that UGT1A1*28 may represent a risk factor for irinotecan toxicity, but this relationship appears to be confounded by other, as yet unidentified, factors.

Summary for UGT1A1. The association between the promoter polymorphism in the UGT1A1 gene and irinotecan-related toxicity is now referenced in the Camptosar label (irinotecan hydrochloride). However, it is difficult to make clear recommendations for the pharmacogenetic screening of UGT1A1 prior to the initiation of irinotecan therapy because other factors, such as dose,[29] seem to confound the relationship between genotype and drug response.

UGT2B7

UGT2B7 gene and function. UGT2B7 is predominately responsible for the glucuronidation of morphine (to form morphine-3-O-glucuronide and morphine-6-O-glucuronide), many NSAIDs, and zidovudine. The UGT2B subfamily consists of individual genes as part of a gene cluster localized on human chromosome 4 at 4q13-q21.[279]

UGT2B7 polymorphisms. An A to T transversion at nucleotide 802 has been described within the UGT2B7 gene. This allele has been designated as UGT2B7*2. Up to 30% of Caucasians may possess

this allele, whereas up to approximately 21% of Japanese possess this allele. Thus, there is a statistically significant difference in these 2 ethnic groups with respect to the prevalence of UGT2B7 polymorphisms.[280] Although the clinical significance of this polymorphism is still under investigation, there are reports in the literature in cancer patients that there may be an allele-specific decreased ratio of morphine and its glucuronides in plasma.[281] Nevertheless, a recent study by Holthe et al[282] failed to show that this polymorphism is responsible for the variation in plasma ratios of morphine and its glucuronide metabolites.

Summary for UGT2B7. There are no current drugs for which compelling evidence suggests that pharmacogenetic screening of UGT2B7 is required prior to initiating therapy. As new drugs are developed that are metabolized largely via UGT2B7, those drugs should be developed with an understanding of the contribution of UGT2B7 pharmacogenetics toward drug disposition and response.

Glutathione-S-Transferases

Glutathione-S-transferases catalyze the nucleophilic attacks of glutathione on electrophilic substrates. In mammals, GSTs function as dimers with subunits of 199 to 244 amino acids. These GSTs are divided into 7 main classes: alpha, mu, pi, sigma, theta, omega, and zeta.

GST polymorphisms. Of the 7 major classes of GSTs, 2 in particular (ie, mu [GSTM] and theta [GSTT]) have been associated with several polymorphisms with functional significance. There are several alleles of GSTM. The protein changes related to these allelic variants range from no change (eg, GSTM3*B and GSTM4*B) to overexpression of the protein (eg, GSTM1*1x2) to no protein (eg, GSTM1*0). In addition, to date, several polymorphisms have been defined for GSTT. Interestingly, GSTT1*0, like GSTM1*0, has a gene deletion, which results in no protein being expressed. There is no current evidence for the primary contribution of GST enzymes toward clearance of marketed drugs.[283]

Summary for GSTs. The importance of GST polymorphisms to pharmacotherapy is still in its infancy. As new drugs are developed for which GSTs represent a major metabolic pathway, those drugs should be developed with an understanding of the contribution of GST genotypes toward drug disposition and response.

DRUG TRANSPORTERS

ATP Binding Cassette Transporters

ATP binding cassette (ABC) transporters are present in tissue barriers and excretory organs where they efflux substances from an organ or the body, often against a concentration gradient (eg, excretion into bile).[284] Due to their role in the efflux of chemotherapeutics and other drugs from their target cells, the ABC transporters were historically referred to as multidrug resistance transporters (MDRs). There are at least 49 ABC transporter genes (http://nutrigene.4t.com/translink.htm). The key ABC transporters involved in the disposition of drugs are ABCB1 (P-glycoprotein or P-gp, MDR1), ABCG2 (BCRP, mitoxantrone resistance protein [MXR]), and the ABCC1-6 family (multidrug resistance protein [MRP]).[285] A summary of genetic polymorphisms of many human transporters can be found at the PharmGkb Web site (http://www.pharmgkb.org/index.jsp).

ABCB1

ABCB1 (P-gp, MDR1) is the most studied drug transporter. It was discovered in the 1970s, and overexpression was shown to be associated with broad chemotherapeutic resistance in tumor cells and thereby be responsible for "untreatable" refractory cancer. It was later found to be expressed in tissue barriers and excretory organs such as the blood-brain barrier, gastrointestinal mucosa, and the liver.[286,287] In the early 1990s, the role of ABCB1 in pharmacokinetic drug-drug interactions began to emerge. It became apparent that ABCB1 not only limited the bioavailability of drugs but also influenced their distribution and clearance.[288] This protein transports a wide variety of hydrophobic molecules.[289] An exact pharmacophore has yet to be defined for this transporter due to its broad substrate specificity. However, its substrates tend to be large, lipophilic compounds with a neutral or positive charge.

ABCG2

ABCG2 (BCRP, MXR) was first identified in a resistant breast cancer cell line.[290] It has been shown to transport flavonoids, steroids, drugs, and sulfate metabolites. ABCG2 is a key transporter involved in limiting the bioavailability of several drugs, concentrating drugs into breast milk, protecting the fetus from drugs in the maternal circulation, and eliminating drugs into the bile.[291] However, its role at the blood-brain barrier appears to be limited relative to the role of P-gp.[290] The most notable drugs known to

be transported by ABCG2 are topotecan, nitrofurantoin, mitoxantrone, and anthracycline.

ABCC Family

The next major family of ABC transporters involved in the transport of xenobiotics and their metabolites is the ABCC family (MRP1-6), and the most studied ABCC family member is ABCC2 (MRP2, cMOAT).[292] ABCC2 is expressed in the canalicular member of hepatocytes, the apical membrane of intestinal enterocytes, and the kidney and brain, although the location of MRP2 within the brain is still controversial. It is known to transport organic anion conjugates (sulfates and glucuronides) and conjugated bilirubin. It also transports vincristine and vinblastine and plays a role in multidrug resistance. Although many drugs are known to interact with ABCC2 in vitro, there is little evidence that ABCC2 plays a major role in the pharmacokinetics of any xenobiotics in vivo. The transporter is believed, however, to play a key role in metabolite excretion, especially in rats, and may have an important role in protecting the liver from toxicity. Mutations in this transporter can cause Dubin–Johnson syndrome, an autosomal recessive disorder characterized by mild conjugated hyperbilirubinemia, which suggests that inhibition of MRP2 by drugs may also cause hyperbilirubinemia.

The importance of other ABCC family members in drug disposition is still being clarified. For example, ABCC1 may have a role in protecting the central nervous system, ABCC3 transports bile acids and conjugates from liver to blood, and ABCC4, 5, and 8 are known to transport nucleoside analogs.[292]

Clinically Significant Polymorphisms of the ABC Transporters

ABCB1

Although the role of ABCB1 in the disposition of drugs has become clearer in the past 10 years, the impact of polymorphisms on its function remains to be clarified. The pharmacogenetics of ABCB1 and its clinical implications have been extensively reviewed.[293-295] To date, more than 100 genetic variants have been reported in the ABCB1 gene, of which the silent mutation in exon 26 (C3435T) and the nonsynonymous mutation in exon 21 (G2677T/A) are of particular interest because they are associated with differences in expression and/or function. The C3435T SNP in ABCB1 has been associated with susceptibility to renal cell carcinoma, Parkinson's disease, inflammatory bowel disease, refractory epilepsy, and response to HIV therapy. Despite the fact that a number of clinical studies have shown a link between the pharmacokinetics of drug and ABCB1 polymorphisms, the findings from many studies have not been consistently reproduced.[296,297] Many of the studies investigated only the C3435T polymorphism, which is in linkage disequilibrium with other variants and has been reported to interfere with mRNA stability.[298] When studies are performed using haplotype analysis rather than SNPs, it is hoped that the true role of ABCB1 polymorphisms in the pharmacokinetics of a drug or endogenous substance will be clarified. In a recent publication, a lower exposure to amlodipine was related to haplotypes of ABCB1.[299] Currently, application of ABCB1 polymorphism analysis to clinical studies is very limited due to the lack of a strong association between specific alleles and drug disposition. Nevertheless, ABCB1 genotyping should be considered in cases of PK variability for known ABCB1 substrates. In light of the role of ABCB1 in the blood-brain barrier, ABCB1 genotyping in relation to efficacy or adverse events should also be considered for ABCB1 substrates, even in the absence of PK variability. It is of interest that there are no reports of humans being deficient in ABCB1 function, suggesting that ABCB1 activity may be an essential function, which is in contrast to the well-documented abcb1 alleles in dogs and mice that result in natural abcb1-deficient animals.[300,301]

ABCC2

In vitro studies have identified variants of ABCC2 with functional consequences such as increased activity and changes in affinity.[302] A recent study by Meier et al[303] investigated the effect of polymorphisms on transporter expression in the human liver. High liver expression of ABCC2 was significantly correlated with 2 variants (3600T>A and 4581G>A). A Dubin-Johnson patient in whom the highly conserved arginine was replaced by glycine at position 412 had unusually high methotrexate plasma concentrations and a 3-fold reduction in methotrexate elimination rate, resulting in reversible nephrotoxicity.[304] A more common polymorphism in ABCC2, (27C>T), has been correlated with a 2-fold increase in exposure to methotrexate, resulting in an increased need for folate rescue in these patients.[305] Although Niemi and colleagues[306] indicated that there was no correlation between ABCC2 polymorphisms and pravastatin pharmacokinetics, they have recently published evidence that the 1446C>G polymorphism caused a decrease in exposure to pravastatin.[307] A possible reason for the discrepancy is the low frequency of the 1446C>G genotype in the first study. The 1446C>G variant has increased activity in

vitro, which may translate to decreased pravastatin exposure in vivo. The ABCC2*2 haplotype has been correlated with a decreased frequency of diarrhea in patients treated with irinotecan.[308] The ABCC2*2 haplotype has decreased activity, and the resultant decreased biliary excretion of irinotecan is thought to be the reason for the correlation with diarrhea, although the ABCC2*2 haplotype was not related to differences in the plasma pharmacokinetics of irinotecan. This finding will need to be confirmed in larger studies. The effect of variants of ABCC2 on the disposition of drugs has only been shown for methotrexate when it was given at relatively high doses. Whether variants of ABCC2 will have a broad impact on the disposition of drugs has yet to be elucidated.

ABCG2

The ABCG2 gene has been screened for genetic variations in 11 ethnic populations, and more than 40 SNPs have been identified.[309] The ABCG2 421 C>A allele is associated with low ABCG2 expression levels and altered sensitivity to several anticancer drugs in vitro. This allele is carried by ~35% in the Japanese and Chinese populations.[310] Diflomotecan plasma levels were significantly higher in subjects with the C421A genotype.[311] Similarly, in a study in 14 Chinese healthy volunteers, exposure to rosuvastatin was significantly lower in the CC genotype group compared with the CA and AA genotype groups, after controlling for SLCO1B1 and CYP2C9 genetic polymorphisms.[310] These 2 studies demonstrate the clinical importance of the ABCG2 C421A genotype. ABCG2 is also believed to play an important role in the elimination and distribution of irinotecan. A recent report has correlated an upstream deletion (ABCG2-19572-19569 CTCA deletion) with the relative extent of conversion of irinotecan to its active metabolite, SN-38.[312] Patients with the wild-type CTCA genotype had a greater relative extent of conversion of irinotecan to SN-38 compared with those with the heterozygous or homozygous CTCA-deleted genotype. The CTCA-deleted genotype may be responsible for lower expression of the ABCG2 protein, although this has yet to be documented. Because all studies to date have been completed with relatively small populations, larger studies will be required to elucidate the true role of ABCG2 variants in drug distribution.

Solute Carrier Transporters

Solute carrier (SLC) transporters are found throughout the body and play a key role in cellular homeostasis and distribution of nutrients.[313] The SLC transporters consist of channels, facilitated transporters, and active transporters. The Human Genome Organization (HUGO) Nomenclature Committee Database includes more than 40 transporter families of the SLC gene series (http://www.bioparadigms.org/). The families of solute carriers of emerging importance in drug disposition are SLC22 (organic cation transporters [OCT] and organic anion transporters [OAT]), SLCO (formally SLC21, the organic anion-transporting polypeptides [OATPs]), and SLC15 (the peptide transporters).

SLC22A1-3 (organic cation carriers, OCT1-3) transport cations down an electrochemical gradient and therefore, depending on the established gradient across the membrane, translocate substrates either into or out of the cell.[314-316] SLC22A1 (OCT1) is located in the liver sinusoidal membrane and, to a lesser extent, in the small intestine; SLC22A2 (OCT2) is located in the basolateral membrane of the proximal tubules and in neurons; and SLC22A3 (OCT3) is more ubiquitous and located in the liver, skeletal muscle, placenta, kidney, heart, lung, and brain. They have a broad substrate overlap, and the substrates include metformin, phenformin, cimetidine, acyclovir, and quinidine.

The organic anion transporters SLC22A6 (OAT1), SLC22A7 (OAT2), SLC22A8 (OAT3), SLC22A11 (OAT4), and SLC22A10 (OAT5) are known to transport a variety of drugs, including NSAIDs (acetaminophen, ibuprofen, indomethacin, naproxen, salicylate), cephalosporin antibiotics, antivirals (acyclovir, cidofovir, ganciclovir), and methotrexate.[317,318] SLC22A6, SLC22A8, and SLC22A11 are located in the kidney tubules; SLC22A6 and A8 are on the basolateral membrane of the proximal tubules; and SLC22A11 is on the luminal membrane of the proximal tubules. SLC22A7 and SLC22A10 are located in the liver, with SLC22A7 expressed on the sinusoidal membrane; the location and function of SLC22A10 are not known.

The SLCO (OATP, formally SLC21) family contains a large number of transporters that are expressed throughout the body.[319,320] The family's natural substrates include bile salts, thyroid hormone, and prostaglandins. However, some of the forms also transport benzlypenicillin and statin drugs. The most prominent member of the family is OATP1B1 (SLCO1B1, OATP-C), which has been identified as a key mechanism for the transport of pravastatin, rosuvastatin, pitavastatin, simvastatin, fluvastatin, atorvastatin, lovastatin, and cerivastatin across the hepatic sinusoidal membrane with greater significance for hydrophilic statins.[320,321] SLCO1B1

is located on chromosome 12.[319] SLCO1B3 (OATP8) also encodes a liver-specific uptake transporter with similar substrates to SLCO1B1, with the one notable difference that digoxin is a substrate of SLCO1B3 but not of SLCO1B1. SLCO1A2 (OATP-A) and 2B1 (OATP-B, OATP-RP2) are expressed in the liver. However, members of this family of uptake transporters are also present in the central nervous system (SLCO1A2) and intestinal tract (SLCO2B1). Their roles, along with a number of SLCO family members, in drug disposition are still being clarified.

There are 2 predominant peptide transporter genes, SLC15A1 (PEPT1) and SLC15A2 (PEPT2).[322] SLC15A1 is primarily expressed in the intestine and, to a lesser extent, in the kidney. In contrast, SLC15A2 is expressed in the kidney and is responsible for reabsorption of di- and tripeptides from the urine. SLC15A2 is also expressed in the central nervous system and lung. In addition to di- and tripeptides, the peptide transporters transport some cephalosporin antibiotics, angiotensin-converting enzyme (ACE) inhibitors, renin inhibitors, and peptide-like prodrugs. The genes are highly conserved across species.[323]

Clinically Significant Polymorphisms of the SLC Transporters

SLCO1B1

Sixteen SNPs and haplotypes have been identified for SLCO1B1. Comparative studies of functionally relevant SNPs for this transporter in African and European Americans revealed large interethnic differences in allele frequencies, which differed significantly in activity when tested in vitro.[324] The functional significance of the polymorphisms of SLCO1B1 on the disposition kinetics of pravastatin has been demonstrated, indicating the potential impact of SLCO1B1 polymorphisms on certain classes of drugs.[306,325,326] Polymorphisms in SLCO1B1, which cause a reduced uptake of pravastatin into hepatocytes, have been associated with decreased drug effectiveness, leading to a smaller inhibitory effect on cholesterol synthesis.[327] However, in a larger study of statins, no association between SLCO1B1 SNPs and efficacy was observed.[22] This larger study did not look at haplotype associations, which may mean that the data from the smaller study could be a spurious result or haplotypes are necessary for association. Although all these studies were relatively small, they consistently showed a significant effect of SLCO1B1 nonsynonymous variation Val174Ala (c.521T>C) on pravastatin pharmacokinetics. The pharmacokinetics of pitavastatin, but not pitavastatin lactone, have also been associated with an SLCO1B1 allelic variation.[328] Katz and colleagues[17] have shown an association of OATP1B1 transport phenotypes on both single-dose and steady-state pharmacokinetics of atrasentan. The 521 T>C polymorphism has also been associated with increased exposure to repaglinide and nateglinide.[329,330] A small clinical study has also shown that the OATP1B1 phenotype appears to play a role in fexofenadine pharmacokinetics.[331] This observation has not been replicated in a larger study or by another laboratory; therefore, its significance is unclear.

SLC15A and SLC22

Although the functional consequences of polymorphisms of PEPT1,[332,333] PEPT2,[334] OATs,[320,335,336] and OCTs[337-339] have been described in vitro, exempt from OCT1, no studies reported in the literature to date clarify the clinical implications of this observation. In contrast, OCT1 loss-of-function variants have recently been shown to affect hepatic metformin uptake and oral glucose tolerance of healthy volunteers after metformin treatment.[340]

Summary of ABC and SLC transporter polymorphisms. Although there is strong evidence for the role of transporters in drug disposition, the application of genotyping to clinical studies is limited at this time. For ABCG2 and SLCO1B1, there is strong evidence supporting genotyping for selected alleles if an investigational drug is shown to be a substrate for these transporters and there is notable variability in the clinical pharmacokinetics of the drug or the drug has a narrow therapeutic index. For other transporters, such as ABCB1, ABCCs, SLC15A, and SLC22, the clear application of PGx to understand pharmacokinetics and dynamics is lacking. Therefore, it is recommended that genotyping for ABCG2 and SLCO1B1 polymorphisms should be conducted when appropriate, and genotyping for other transporter genes should remain a research activity to clarify the significance of their polymorphisms on drug disposition.

GENOTYPE AND PHENOTYPE CORRELATIONS

Correlating an individual's genotype with the in vivo activity of a given DME or transporter can be difficult. Although in most cases, there is a good correlation between genotype and phenotype, there are examples where genotype and phenotype are not correlated, often due to factors that can affect the regulation

or activity of the polymorphic gene or other nongenetic influences on phenotype. Such factors include disease, age, diet, gut flora, and coadministered drugs. In these instances, correlations between genotype and phenotype that exist in the "normal" state can be lost when enzyme induction or downregulation occurs. Furthermore, there are a number of examples where genotypic differences (eg, different alleles) exist but do not affect the phenotype.[341] Such associations may not be sufficient for applications such as diagnostics but may still be useful for other purposes such as informing on the mechanism of drug metabolism and disposition. Correlations between genotype (a static phenomenon) and phenotype (a dynamic phenomenon) and their relevance to drug therapy should always be made with caution.

OVERALL CONCLUSIONS

The authors of this article recommend prudent use of genotyping activities for drug-metabolizing enzymes and transporters as drug candidates proceed through successive stages of drug development. Although general recommendations for the genotyping of specific ADME genes and alleles are made, this should not exclude the genotyping of other ADME genes/alleles where sufficient rationale exists. Genotype-phenotype relationships for several drug-metabolizing enzymes (eg, CYP2C9, CYP2C19, CYP2D6, UGT1A1) are well established, whereas data for most transporters are still emerging. In the ideal scenario, where new drug application submissions contain information stating the impact of genotype on pharmacokinetics, efficacy, and/or safety, there will be sufficient confidence in the data for inclusion in the prescription label and whether dosing should be targeted according to genotype. As of the time of writing this article, there are no approved drugs in the United States that *require* dosage adjustments according to drug-metabolizing enzyme or transporter genotype, although there are several examples of *recommended* dosage adjustment to optimize safety profiles. As our understanding of the underlying science evolves, and the practice of enzyme and transporter genotyping becomes more commonplace, more prescription labels will contain recommendations, or even requirements, to target dosing according to genotype. This evolving practice should help in our mission to minimize drug-related adverse events, maximize drug efficacy, and better characterize the metabolism of our compounds.

APPENDIX
PHARMACEUTICAL RESEARCH AND MANUFACTURERS OF AMERICA (PhRMA) PGx SURVEY

(Note: Not every company answered every question, so the number of responses may vary with question.)

1. How often has your company collected DNA with consent for ADME-related genotyping in:

	Number of Responses			
Study Type	**Always**	**Usually**	**Sometimes**	**Never**
First in human	2	11	1	
Multiple rising dose	2	12		
Drug-drug interaction	1	11	2	
Special population		8	5	1
Other clin pharm		11	3	
Proof of concept		7	7	
Dose ranging		7	7	
Pivotal		6	8	
Other		4	8	1

2. How often has your company performed ADME-related genotyping in:

	Number of Responses			
Study Type	**Always**	**Usually**	**Sometimes**	**Never**
First in human	1	1	12	
Multiple rising dose	1	1	12	
Drug-drug interaction	1		12	1
Special population		1	11	2
Other clin pharm		1	11	2
Proof of concept			12	2
Dose ranging		1	8	5
Pivotal			10	4
Other			9	5

3a. Has your company used ADME-related genotype(s) in study design?

	Number of Responses	
	Yes	**No**
Inclusion criterion	10	4
Exclusion criterion	10	4

(continued)

APPENDIX (continued)

3b. If so, which genes?

Gene	Number of Responses	Gene	Number of Responses
CYP2D6	11	CYP3A5	1
CYP2C19	7	GSTM1	1
CYP2C9	4	MGMT	1
UGT1A1	4		

4. How often has your company specified ADME PGx analysis in study protocols?

	Always	Usually	Sometimes	Never
Number of Responses		2	12	

5. Breadth of genotyping. Please check whether your company currently genotypes each gene.

	Number of Responses	
Gene	Yes	No
CYP1A2	7	4
CYP2A6	6	5
CYP2B6	6	5
CYP2C8	7	5
CYP2C9	12	1
CYP2C19	13	
CYP2D6	14	
CYP3A4	8	4
CYP3A5	11	3
Other phase I enzyme	6	3
UGT1A1	13	1
TPMT	3	8
Other phase II enzyme	8	3
OATP1B1	7	4
BCRP	7	4
MDR1	9	3
Other transporter	7	5
Other PK-related	7	5

6. Has your company only genotyped when preclinical data indicate a role for the gene in a compound's PK, or do you genotype a broader range of genes?

	With Preclinical Support Only	Broader Range
Number of responses	8	6

7. How often does your company have a written ADME PGx plan or strategy for a compound in development?

	Always	Usually	Sometimes	Never
Number of responses	2	1	9	2

8. Has genotyping been done within your company or outsourced? (OK to check both)

	In-House	Outsourced
Number of responses	13	12

9. How has your company coded samples collected for ADME PGx research? (OK to check more than one)

	Single Coded	Double Coded	Anonymized/ Anonymous
Number of responses	8	10	3

10. Has your company kept/banked DNA beyond the initial period of the clinical trial?

	Yes	No
Number of responses	13	

11. When ADME PGx research has been included in a trial, has it been a required study activity or optional for each subject in? (OK to check both boxes in a row)

	Number of Responses	
Study Type	Yes (Required)	No (Optional)
Phase I studies	12	12
Drug interaction studies	10	8
Phase II studies	4	12
Phase III studies	3	13

12. How often has your company used phenotyping to ensure that genotype-assigned phenotypes are correct?

	Always	Usually	Sometimes	Never
Number of responses			6	8

(continued)

APPENDIX (continued)

13. Has your company combined samples across studies of a single compound to enhance the statistical power of ADME PGx analysis?

	Yes	No
Number of responses	11	1

14. How important has replication of an ADME PGx finding been in your company? (please check only one box)

	Number of Responses
An independent replication has always been necessary	1
An unreplicated result has been used for internal decision making but not in a regulatory submission	7
An unreplicated result based on a known valid biomarker has been used in a regulatory submission	2
An unreplicated result based on another biomarker has been used in a regulatory submission	
PG-PK results have not been used	2

15. How often has the statistical power of ADME PGx analysis entered into study design criteria at your company?

	Always	Usually	Sometimes	Never
Number of responses			9	5

16. Has your company used large-scale (eg, multigene chip-based) exploratory ADME PGx analysis?

	Yes	No
Number of responses	6	8

17. How often has your company used any FDA-approved in vitro diagnostic (UGT1A1 kit or CYP2D6/2C19 chip) for ADME PGx for clinical trial applications?

	Always	Usually	Sometimes	Never
Number of responses			6	8

18. Does your company apply the following standards for human DNA sample collection and generation of human genotype data that might be used in regulatory submissions?

	Number of Responses	
	Yes	No
GCP	14	
GLP	10	3
GMP	2	10

19. Have scientists from your company published original ADME PGx research in peer-reviewed journals?

	Yes	No
Number of responses	8	6

20. Has ADME PGx information been used in decision making at your company?

	Yes	No
Number of responses	11	3

21. Has your company interacted with FDA or other regulators regarding ADME PGx?

	Yes	No
Number of responses	10	3

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