Pharmacogenetics and Pharmacogenomics of Anticancer Agents

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Abstract

Large interindividual variation is observed in both the response and toxicity associated with anticancer therapy. The etiology of this variation is multifactorial, but is due in part to host genetic variations. Pharmacogenetic and pharmacogenomic studies have successfully identified genetic variants that contribute to this variation in susceptibility to chemotherapy. This review provides an overview of the progress made in the field of pharmacogenetics and pharmacogenomics using a five-stage architecture, which includes 1) determining the role of genetics in drug response; 2) screening and identifying genetic markers; 3) validating genetic markers; 4) clinical utility assessment; and 5) pharmacoeconomic impact. Examples are provided to illustrate the identification, validation, utility, and challenges of these pharmacogenetic and pharmacogenomic markers, with the focus on the current application of this knowledge in cancer therapy. With the advance of technology, it becomes feasible to evaluate the human genome in a relatively inexpensive and efficient manner; however, extensive pharmacogenetic research and education are urgently needed to improve the translation of pharmacogenetic concepts from bench to bedside. CA Cancer J Clin 2009;59:42-55. ©2009 American Cancer Society.

Introduction

It has long been observed that interpatient variability in response to medications is associated with a spectrum of outcomes, ranging from failure to demonstrate an expected therapeutic effect to an adverse reaction resulting in significant patient morbidity and mortality, as well as increasing healthcare costs.1,2 Interpatient variation is due, at least in part, to genetics. The term “pharmacogenetics” represents the study of genetic factors that influence response to drugs and chemicals and was first termed in 1959.3 Recently, advances in large genome scale sequencing and improvements in bioinformatic tools in processing large amounts of data have led to the transition of pharmacogenetics to pharmacogenomics, involving studies of the entire spectrum of genes in the human genome.3

The goal of the emerging disciplines of pharmacogenetics and pharmacogenomics (abbreviated jointly as PGx) is to personalize therapy based on an individual’s genotype. To date, the success of PGx has spread across all fields of medicine. Genetic information has been used in the identification of disease risk (eg, the BRCA1 mutation test to evaluate breast cancer risk), choice of treatment agents (eg, CYP2D6 in breast cancer treatment; HLA-B*1502 for carbamazepine), and guiding drug dosing (eg, CYP2C9 and VKORC1 for warfarin dosing, UGT1A1 for irinotecan, and TPMT for 6-mercaptopurine and azathioprine). This is particularly important for chemothera-

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peutic agents, which in general affect both tumor and nontumor cells and thus have a narrow therapeutic index, with the potential for life-threatening toxicity. Medical oncologists and hematologists are striving to individualize cancer treatment in an effort to maximize efficacy and minimize toxicity in patients. Identifying host genetic variations that contribute to drug efficacy and/or the risk of toxicity will provide a means with which to tailor therapy. Genetic variation could explain variations including pharmacokinetics, alterations in activity or expression of the target, or proteins involved in the mechanism of action of the drug. PGx studies of anticancer agents are potentially complicated by somatic mutations in the tumor, although this is unlikely to impact toxicity.

In the scope of anticancer PGxs, genotypic information can encompass, but is not limited to, single nucleotide polymorphisms (SNPs; a change in which a single base in the DNA differs from the usual base at that position), haplotypes (a set of closely linked genetic markers present on one chromosome that tend to be inherited together), microsatellites or simple sequence repeats (polymorphic loci present in DNA that consist of repeating units of 1-6 base pairs in length), insertion and/or deletion and copy number variations (CNVs; genetic trait of differences in the number of copies of a particular sequence present in the genome of an individual), and aneuploidy (a change in the number of chromosomes that can lead to a chromosomal abnormality) and loss of heterozygosity in the tumor. Phenotype is defined as the characteristics determined by genetic, environmental factors or their combination. Phenotype can take many different forms. For example, it could simply be eye color, or it can be a pharmacokinetic finding (eg, a specific metabolite formation), a clinical marker (eg, tumor volume), or a more complicated trait (eg, overall survival after treatment). Interestingly, researchers from different fields may define phenotypes differently. For example, mRNA expression is treated as a phenotype by geneticists because it is a product of DNA; however, clinicians often lump gene and gene products (eg, RNA and protein) together as genetic predictors. A clear definition of phenotype is critical both in conducting and interpreting PGx studies. As an example, when evaluating the genetic contribution to platinum agent-induced toxicity in patients with non-small cell lung cancer, a genetic polymorphism in the ERCC1 gene (C8092A) was found to be associated with an increased risk of grade 3 or 4 gastrointestinal toxicity (defined by the National Cancer Institute Common Toxicity Criteria version 3.0). However, when using overall toxicity or hematologic toxicity as the phenotype of interest, no genotype-phenotype associations were identified. Furthermore, the treatment conditions under which the phenotypes were obtained may affect the interpretation of the genotype-phenotype association. Through a meta-analysis, Hoskins et al demonstrated that the UGT1A1*28 polymorphism is associated with an increasing risk of hematologic toxicities only at medium or high doses of irinotecan.

This review will provide an overview of the progress made in the field of PGx using a five-stage architecture (Fig. 1). Although considerable effort has been applied to identifying gene expression markers that affect drug response, this review will focus mainly on the germline genetic effects on drug sensitivity. Examples will be provided to illustrate the identification, validation, utility, and challenges of these PGx markers with a focus on the current application of PGx knowledge in cancer therapy.

Determining the Role of Genetics in Drug Response

Before conducting a PGx study, it is important to determine whether genetic variation is likely to have a significant impact on the phenotype of interest. Heritability analysis is often used as an approach in genetics to screen for the presence of a heritable trait. The goal of heritability analysis is to determine how much of the variation in phenotype can be attributed
to genetic variance. Heritability measures can range from 0 to 1. A heritability measure close to 1 implies that nearly all variations in the population result from variations in genotypes. A heritability measure close to 0 for a trait implies that nearly all variations are due to environmental causes. A significant heritable component for a given phenotype provides a strong foundation for follow-up genetic analysis.

To estimate the heritability of complex human phenotypic traits, twin studies have been commonly used because identical (monozygotic) twins share (approximately) the same genotype whereas nonidentical (dizygotic) twins do not. If monozygotic and dizygotic twins share similar environments, the differences in the phenotype of interest may be expected to be due to differences in genotype. Heritability can also be estimated in large pedigrees, which compares the variances within family pedigrees with those among the population.

Clinically, alcoholism and cigarette smoking have been found to be strongly heritable. Furthermore, Faraday et al evaluated the ex vivo platelet function before and after aspirin ingestion in 1880 subjects. These platelet function phenotypes were grouped as cyclooxygenase-1 (COX-1)–dependent if arachidonic acid was the platelet stimuli or if a thromboxane metabolite was measured. All other measures of platelet function testing were deemed COX-1–independent (eg, whole blood aggregation), which represents residual platelet function after aspirin exposure. The authors performed heritability analyses on both COX-1–dependent and COX-1–independent phenotypes and found phenotypes indirectly related to COX-1 were strongly and consistently heritable in both white and African American populations, whereas COX-1–dependent phenotypes were only heritable in the white population. This provides a strong genetic basis for the adequacy of platelet suppression by aspirin. Luciano et al demonstrated that the heritability of coffee–attributed sleep disturbance was approximately 0.4, which suggest that 40% of the overall variation in sleep disturbance is due to genetic factors. However, a heritability study of toxic drugs (eg, most anticancer agents) is not possible in unaffected family members. Instead, an approach using cell lines from large pedigrees has been used for several cytotoxic agents, including bleomycin, cisplatin, docetaxel, fluorouracil (5-FU), and daunorubicin. PGx research can either focus on known SNPs (ie, genotyp-
ing) or the identification of new SNPs in candidate genes (ie, sequencing).

Although candidate gene approaches have had reasonable success in identifying genetic variants that are important in specific phenotypes (eg, drug metabolism, the mechanisms of action of drugs), clinically relevant phenotypes, such as drug toxicity or response, are more complex and therefore likely to involve multiple genes or pathways. Thus, instead of searching for a “dramatic genetic effect” produced by one gene, it is more realistic to consider a group of genetic variants, each with a moderate effect, which together result in an overall genetic effect in drug efficacy or toxicity. Furthermore, to our knowledge, the functions of many of the 20,000 to 25,000 genes in the human genome have not been well studied to date. To resolve these issues, a broader approach has been used.

Genome-wide Approach

Genome-wide approaches in PGx refer to the global study of genetic variations within the human genome for their effects on drug treatment. The hypothesis is that any genetic variant in the human genome can contribute to variation in drug effect. Therefore, these studies are not biased toward current knowledge of gene function and have the potential to identify multiple genetic variants that contribute to complex clinical traits. Recent advances in genomic technology such as genome scale microarray genotyping platforms (which identify SNPs and CNVs), microarray-based comparative genomic hybridization (array CGH; which detects chromosome copy number changes), and transcriptional level gene expression platforms (which measure mRNA level gene expression), along with the development of software to accomplish the analysis of these large data sets, have allowed researchers to perform genome-wide association study (GWAS) between genetics and phenotypes.

GWAS is an examination of genetic variations across the human genome, designed to identify genetic associations with observable traits such as blood pressure or weight, or with/without a disease or condition. As with all PGx studies, a clearly defined phenotype of interest is essential. Phenotypes evaluated in GWAS can be either qualitative/categoric factors (response/no response, alive/dead) or quantitative/continuous measures (tumor volume, minimal residual disease). Analyzing genome scale genetic data requires large computational capacity and generally has a high risk of false discovery. Given a P value of .05, one can expect 5 false-positive findings after 100 tests. When 1 million tests are performed, a large number of positive findings can be false discoveries. Many statistical methods have been applied to reduce the false discovery rate, including using stringent P value cutoff values (eg, Bonferroni correction). Another substantial challenge associated with GWAS is the interpretation of statistically significant findings.

Several national and international collaborative efforts have provided large amounts of genetic and expression information from different samples including human lymphoblastoid cell lines (LCLs; International HapMap), tumor cell lines (NCI-60; available at: http://dtp.nci.nih.gov/docs/misc/common_files/cell_list.html; Accessed November 20, 2008), blood, and tumor biopsy samples. These international efforts have all had the common principle of public data sharing. LCLs were derived from peripheral blood B-lymphocytes that have undergone in vitro Epstein-Barr virus transformation to result in immortalization. In particular, the International HapMap LCLs were derived from individuals with no apparent disease. Using LCLs, Huang et al developed a “triangle approach” that integrates SNP genotype, gene expression, and in vitro cellular sensitivity to drugs to identify genetic variants that contribute to sensitivity to chemotherapeutic agents through their effect on gene expression. The statistical associations of the triangle approach result in a final set of SNPs that comprise a “pharmacogenetic signature” that determines susceptibility to chemotherapeutic-induced cytotoxicity. A follow-up multivariate model was performed to limit the number of genetic variants identified through this model that explain the observed human variations in cellular sensitivity to chemotherapy. The pharmacogenetic signature SNPs are interesting candidates for both follow-up clinical and bench studies. These studies represent a new paradigm in studies of drug pharmacology by evaluating the entire genome for genetic variations that may influence the expression of genes that are important in drug sensitivity. Because the triangle approach involves multiple testing, which is inherently associated with false discovery, replication, as with all GWAS, is imperative.
Given the similar goal and complementary nature of candidate gene and genome-wide approaches, one can imagine that a combined candidate and genome-wide approach would likely provide a list of genetic variants that contribute to complex phenotypes that can be tested in the clinical trials.

Validating Genetic Markers

Replication and Validation

Replication is an important principle in all genetic studies. This is particularly important when a genome-wide approach is used to obtain potential genetic markers. Several large GWAS that focused on identifying disease risk-associated genetic markers have set good examples in using replication samples. For example, a GWAS conducted in 14,000 cases of 7 common diseases and 3000 controls confirmed many previously identified disease-associated loci and found compelling evidence that some loci confer risk for more than 1 of the diseases studied.20 Furthermore, disease-related loci have also been identified and replicated in patients who have prostate cancer,24 bipolar disorder,25 and colorectal cancer.26

If a genotype-phenotype association is replicated, functional studies are usually performed to gain a better understanding of the nature of the association and identify the causative variant. There are many reasons for failing to replicate the initial genotype-phenotype association. It can be that the initial association occurred by chance, or the genetic effect may be smaller than hypothesized based on prior studies, potentially due to factors such as disease status, comorbidity, the administration of other drugs, or patients’ demographic characteristics. Unless there are distinguishable characteristics between the initial screen data set and the subsequent replication data set(s), failure in replication suggests the particular variation is not a sole candidate marker for a large-scale follow-up study.

Large genetic effect sizes as well as well-defined phenotypes are critical in the success of clinical validation. In addition, consistency of potential confounding factors (eg, patients’ demographic characteristics) between the discovery set and validation data set is important. The design of a clinical validation study needs to consider such variabilities to test the hypothesis generated from the initial screening data set. Exploring the clinical implication of genetic predictors in other populations will broaden the utility of PGx in practice.

Clinical Utility Assessment

Once validated, PGx markers can be measured before the initiation of therapy. The information will better inform prescribers as to whether the patient is at an increased risk for nonresponse and/or developing drug-associated toxicities, and can therefore guide their choices of treatment/dose to the individual patient based on the drugs’ therapeutic index. Ideally, an accurate, rapid genotyping assay is required for fast turnaround time. Large prospective clinical studies are also required to provide guidance regarding alternative treatments or dosages. Lastly, the education of prescribers, payers, and patients is essential.

Practical Genotyping

A genotyping assay needs to be specific to the genetic variants of interest and sensitive in identifying the causal allele. Genotyping is generally performed by commercial and academic reference laboratories, as well as by a growing number of hospital laboratories. These assays vary from tests for a single genetic variation (UGT1A1 promoter polymorphism, Mayo Medical Laboratory27; TaqMan SNP Genotyping Assays, Applied Biosystems [available at: https://products.appliedbiosystems.com/ab/en/US/adirect/ab?cmd=catNavigate2&catID = 602849; Accessed November 20, 2008]; GSTM1 CNV, Applied Biosystems [available at: https://products.appliedbiosystems.com/ab/en/US/adirect/ab?cmd=catNavigate2&catID = 603644; Accessed November 20, 2008]), for series of genes (CYP2D6 and CYP2C19 on AmpliChip CYP450 Test, Roche Diagnostics),28 for hundreds to thousands of gene variants (Genome-wide Human SNP Array 6.0, Affymetrix; Agilent Human Genome CGH Microarray 244A, Agilent; and Illumina 550K Infinium Human-Hap550 SNP Chip, Illumina), or for the entire human genome (23andMe).29 A list of laboratories and clinics that perform genetic tests and consultations can be found on a National Institutes of Health-funded website (GeneTests),30 although only a small minority of genetic tests listed on this site are pharmacogenetic tests. In order for this genetic information to be clinically useful, it must be available within a timeframe that is relevant for the therapeutic decisions it is intended to guide.
In addition to genotyping, gene expression profiling and enzyme activity tests (phenotyping assays) may be used as surrogates for genetic variability. For example, in the evaluation of predictive markers for drug sensitivity, Holleman et al generated gene expression prediction markers for chemoresistance in 173 children with acute lymphoblastic leukemia (ALL). These prediction markers were confirmed in an independent cohort of 98 ALL patients treated at a separate institute. More recently, Potti et al developed gene expression signatures that predict sensitivity to chemotherapeutic drugs using NCI-60 cell lines. Each signature was validated in an independent set of cancer cell lines. The authors further demonstrated that many of these signatures appear to predict clinical response in patient populations. In clinical practice, estrogen receptor, progesterone receptor, and HER-2/neu status have been commonly used as predictors of breast cancer prognosis. A diagnostic assay, OncoType DX, quantifies the likelihood of breast cancer recurrence and assesses the benefit from chemotherapy in women with newly diagnosed, early stage breast cancer. This test has been recommended in the 2007 Update of Recommendations for the Use of Tumor Markers in Breast Cancer from the American Society of Clinical Oncology and the National Comprehensive Cancer Network (NCCN) 2008 Clinical Practice Guidelines in Oncology Breast Cancer (available at: http://www.nccn.org/professionals/physician_gls/PDF/breast.pdf; Accessed November 20, 2008). It is worth mentioning that all expression prediction markers are currently generated in neoplastic cells. To the best of our knowledge, germline gene expression signatures have not been extensively studied as cancer PGx markers.

For enzyme activity tests, examples include the thiopurine S-methyltransferase (TPMT) activity test in red blood cells, which is used for guiding the prescription of 6-mercaptopurine or azathioprine, and the CYP3A activity test using midazolam or the erythromycin breath test (which measures liver CYP3A4 catalytic activity) to evaluate potential drug-drug interactions (eg, between ketoconazole and cyclosporine). The advantage of testing gene expression or enzyme activity is that it theoretically would have a higher correlation with clinical phenotypes, assuming that the assay is well validated. However, gene expression may change after drug exposure and measurement of enzyme activity before every treatment adds additional cost and time, and is unlikely to be cost-effective. Conversely, an individual’s germline DNA sequence remains unchanged over a person’s lifetime, and there is no need to reanalyze.

Interpretation of the Data: Healthcare Professional Education

The complexity of human genetics makes the interpretation of single-assay results difficult. Genetic variations that contribute to drug effect can take on different forms. They could be a single SNP, a haplotype (a set of SNP combinations), or a gene CNV (gene deletion or duplication). Different ethnic populations may have different variations in allele frequency. Furthermore, the effect of any specific genetic variant will vary by drug. Therefore, the education of prescribers regarding PGx is a major challenge.

Evidence-based PGx Dosing

Many clinicians acknowledge the importance of genetics in drug response and are enthusiastic about using genetic information to guide therapy. Although our knowledge of the relation between specific genetic variants and clinical outcomes is increasing, we know far less regarding how to individualize therapy. However, if PGx studies are routinely incorporated into large (ie, phase 3) clinical trials, we will gain knowledge concerning the predictive value of PGx, which will eventually enable the individualization of therapy.

Pharmacoeconomic Impact

PGx is expected to improve patients’ treatment outcomes by increasing efficacy and decreasing toxicity, and eventually reducing overall healthcare costs. Given the high incidence of cancer- and treatment-associated toxicity as well as the cost of treating cancer and protecting patients from toxicity, it is particularly promising to demonstrate a pharmacoeconomic benefit using PGx testing. With the increasing number of PGx findings, continuing education for prescribers and payers concerning updated PGx information is critical. Both retrospective and prospective trials evaluating the pharmacoeconomic impact of PGx testing in cancer therapy will likely provide answers for policy making in the incorporation of PGx testing into clinical practice; however, one should keep in mind that it will not be feasible to conduct randomized trials of each and every diagnos-
tic test, and the economic value of such tests can be modeled using decision analysis techniques.

Examples

Tamoxifen

Tamoxifen, a selective estrogen receptor modulator, has been successfully used in the treatment of breast cancer for more than 30 years and has been approved for breast cancer prevention since 1998. Until recently, clinically useful predictors (other than the presence of estrogen and progesterone receptors) that identify those individuals most likely to benefit from tamoxifen therapy or develop toxicity have been lacking. Recent studies have identified allelic variations in CYP2D6 to be an important determinant of tamoxifen’s activity (and toxicity), because CYP2D6 is responsible for the formation of tamoxifen’s active metabolite.

CYP2D6 is responsible for the metabolism of 25% of all drugs currently on the market that undergo metabolism and its genetic polymorphism significantly affects the metabolism of approximately 50% of those. To date, more than 71 different functional CYP2D6 gene variants have been described (available at: http://www.cypalleles.ki.se/cyp2d6.htm; Accessed November 20, 2008), and these are divided into alleles causing abolished (null), decreased, normal, and ultra-rapid enzyme activity. The most important null alleles are CYP2D6*4 (splice defect) and CYP2D6*5 (gene deletion), whereas the most common alleles with severely reduced activity are CYP2D6*10, CYP2D6*17, and CYP2D6*41 (splicing defect). The CYP2D6 gene is subject to many CNVs. A detailed summary of CYP2D6 genetic variations and their functional consequences can be found in a recent review by Ingelman-Sundberg et al. The CYP2D6 alleles are subject to very significant interethnic differences in which poor metabolizers (the 2 null allele carriers) are found mainly in Europe and ultra-rapid metabolizers (individuals having more than 2 normal CYP2D6 alleles) are found in North Africa and Oceania; intermediate metabolizers (carriers of a reduced functional allele) are to a great extent located in Asia due to the high prevalence of the CYP2D6*10 allele.

It has been shown that tamoxifen is metabolized by CYP2D6 to 4-hydroxy-tamoxifen and endoxifen, which exhibit approximately 100 times greater affinity for the estrogen receptor than the parent drug, tamoxifen. Goetz et al reported a shorter recurrence-free survival time and lower disease-free survival rate in tamoxifen-treated women who were homozygous for the null allele CYP2D6*4 (poor metabolizers) compared with women who were heterozygous or homozygous for the wild-type allele (intermediate or extensive metabolizers). These homozygous variant carriers also experienced less severe hot flashes when compared with wild-type allele carriers. The same group of authors estimated that women who were poor CYP2D6 metabolizers had the highest risk of breast cancer recurrence (hazards ratio of 3.12; \( P = .007 \)). CYP2D6 metabolism, as measured by genetic variation and enzyme inhibition, was found to be an independent predictor of the risk of breast cancer recurrence in postmenopausal women receiving tamoxifen for the treatment of early breast cancer. These findings were confirmed by several other groups. In addition, breast cancer patients in a Japanese population who carried the CYP2D6*10 allele were found to have a significantly higher incidence of disease recurrence with adjuvant tamoxifen monotherapy. Furthermore, medications that decrease CYP2D6 activity, such as antidepressants, were also shown to decrease the efficacy of tamoxifen treatment. These research findings led an advisory committee of the US Food and Drug Administration (FDA) to unanimously recommend a label change for tamoxifen in 2006 (available at: http://www.fda.gov/ohrms/dockets/ac/06/briefing/2006-4248B1-01-FDA-Tamoxifen%20Background%20Summary%20Final.pdf; Accessed November 20, 2008), including mention of CYP2D6 genotype testing as an option for women before they are prescribed tamoxifen. However, the FDA label change recommending CYP2D6 genotype testing before tamoxifen treatment is still pending. Although we believe that the overall data support this proposed label change, prospective studies are warranted.

At the same time, inferring CYP2D6 phenotype from genotype is increasingly challenging, considering the growing number of alleles and their range of activity. Gaedigk et al proposed an “active score” system that categorized individuals of white or African American heritage into different groups based on the metabolism profiles of 672 subjects for dextromethorphan, a CYP2D6 probe drug, over 25
CYP2D6 allelic variants.53 Furthermore, the Ampli-Chip technology allowed for the identification of the CYP2D6 genotype and reported individuals as poor metabolizers, intermediate metabolizers, extensive metabolizers, or ultra-rapid metabolizers. This test was approved by the US FDA on December 24, 2004 and has been adapted by several commercial genotyping companies (eg, DNAdirect).54

To our knowledge, with the exception of ongoing clinical trials (available at: http://www.clinicaltrial.gov; Accessed November 20, 2008), CYP2D6 genotyping has not been routinely incorporated into clinical practice. However, given the broad spectrum of CYP2D6 substrates, the relatively high frequency of CYP2D6 genetic variations, and their confirmed role in multiple drug treatments, it would not be surprising if CYP2D6 genotyping were gradually adopted into clinical practice. Interestingly, a recent modeling analysis suggested that CYP2D6 genotyping may be used for determining the optimal adjuvant endocrine therapy for postmenopausal breast cancer patients. In this analysis, 5-year disease-free survival outcomes were found to be similar or perhaps even superior with tamoxifen compared with aromatase inhibitors.55

Based on their findings, the authors concluded that endocrine therapy tailored to the CYP2D6 genotype could be considered for women who are newly diagnosed with breast cancer, particularly those who have concerns about either the risk of disease recurrence after 6-mercaptopurine therapy, the majority of patients who are at greatest risk for severe toxicity can be identified and decisions regarding the need for dose reductions or other modifications can be made before the patient is exposed to the drug.

In 2003, a US FDA advisory committee recommended the addition of pharmacogenetic information concerning TPMT polymorphisms and treatment toxicity to the drug label for 6-mercaptopurine. This led to changes to the label for 6-mercaptopurine in 2004, with TPMT testing and dosage recommendations provided for TPMT-deficient patients (available at: http://www.fda.gov/medwatch/SAFETY/2004/jul_PI/Purinethol_PI.pdf; Accessed November 20, 2008).59 As 1 of the classic PGx examples in cancer therapy, TPMT activity in human beings was found to be trimodally distributed,33 with approximately 90% of individuals having high activity, 10% having intermediate activity, and 0.3% having low or undetectable enzyme activity. TPMT activity has been shown to correlate with 6-mercaptopurine toxicity and therapeutic efficacy;60 the higher the TPMT activity, and therefore less formation of the active TGNs, the less 6-mercaptopurine toxicity and the higher risk of disease recurrence after 6-mercaptopurine therapy.61,62

To the best of our knowledge, PGx studies have identified at least 21 nonsynonymous variations in TPMT to date, 17 of which were shown to have reduced TPMT activity.63,64 Among these genetic variations, TPMT*2, TPMT*3A, and TPMT*3C account for greater than 90% of cases with low or intermediate TPMT enzyme activity.59 Therefore, by genotyping patients for these 3 variants before 6-mercaptopurine therapy, the majority of patients who are at greatest risk for severe toxicity can be identified and decisions regarding the need for dose reductions or other modifications can be made before the patient is exposed to the drug.

6-mercaptopurine

6-mercaptopurine is commonly used for the treatment of ALL. It is a prodrug that is activated by hypoxanthine guanine phosphoribosyl transferase to 6-thioguanine nucleotides (TGN), which are incorporated into DNA. TPMT inactivates 6-mercaptopurine by S-methylation and thereby decreases the formation of active TGN in hematopoietic tissues (available at: http://www.pharmgkb.org/do/serve?objIdA2040&objCls=Pathway; Accessed November 20, 2008).59 As 1 of the classic PGx examples in cancer therapy, TPMT activity in human beings was found to be trimodally distributed,33 with approximately 90% of individuals having high activity, 10% having intermediate activity, and 0.3% having low or undetectable enzyme activity. TPMT activity has been shown to correlate with 6-mercaptopurine toxicity and therapeutic efficacy;60 the higher the TPMT activity, and therefore less formation of the active TGNs, the less 6-mercaptopurine toxicity and the higher risk of disease recurrence after 6-mercaptopurine therapy.61,62

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Based on a recent study, TPMT genotyping has been assessed as being cost-effective in children with ALL. After considering TPMT genotyping costs, estimates of the frequency of TPMT deficiency, rates of thiopurine-mediated myelosuppression in TPMT-deficient individuals, and myelosuppression-related hospitalization costs in 4 European countries, the study found that the cost per life-year gained by TPMT genotyping in ALL patients was €2100, based on genotyping costs of €150 per patient.65

Currently, pretherapy TPMT genotyping and activity tests are only routinely ordered at a limited number of academic centers,66 one of which is St. Jude Children’s Research Hospital. Children’s Oncology Group ALL trial protocols include TPMT genotyping when persistent myelosuppression is observed. A similar relation between TPMT genotype and toxicity has been demonstrated for azathioprine,67 which has led to the use of genotype-guided dosing by gastroenterologists using this antimetabolite for patients with inflammatory bowel disease.68 It is likely that there is more use of this particularly test in gastroenterology than oncology, because the severe neutropenia associated with TPMT deficiency can be avoided in this clinical context. Furthermore, the limited utilization of TPMT genotyping could be due to different hospital policies or other practical issues (eg, genotyping capacity and slow turnaround time). Comprehensive evaluations regarding cost-effectiveness in different populations would be helpful.69 In addition, patients who share the same TPMT genotypes still exhibit considerable variations in their response to 6-mercaptopurine, suggesting that other unidentified genetic variations may contribute to the toxicity and response of 6-mercaptopurine.

**Irinotecan**

Irinotecan is commonly used for the treatment of patients with colorectal and lung cancers. However, up to 30% of patients experience unacceptably severe diarrhea or neutropenia, depending on the dose and schedule used.70 The prodrug irinotecan is converted by carboxylesterases to a more active metabolite, SN-38. UDP-glucuronosyltransferase 1A1 (UGT1A1) is the major UGT1A isoform that conjugates SN-38 to an inactive SN-38 glucuronide71; however, genetic variations in other glucuronosyltransferases (eg, UGT1A7 and UGT1A9)72-74 and transporters (eg, ABCB1, ABCC2, and ABCG2)75-77 have also been suggested to contribute to the variability in irinotecan pharmacokinetics and toxicity.

In white populations, the UGT1A1*28 polymorphism (the presence of an additional TA repeat in the TATA box of the UGT1A1 promoter [TA6]) is the most common variant associated with Gilbert syndrome, a benign form of familial hyperbilirubinemia.78 The insertion of an additional repeat (TA7) is associated with a decrease in UGT1A1 expression and the consequently decreased glucuronidation of its targets.78 The association between UGT1A1*28 and irinotecan toxicity was first demonstrated by Iyer et al,71 and has been confirmed in multiple studies, although the impact of this polymorphism is highly dependent on the irinotecan dose administered.6

Based on PGx evidence, the US FDA has approved the addition of a warning to the irinotecan label (available at: http://www.fda.gov/medwatch/SAFETY/2005/jun_PI/Camptosar_PI.pdf; Accessed November 20, 2008), as well as the marketing of the Invader UGT1A1 Molecular Assay (Third Wave Technologies) for the detection of UGT1A1*28.79 The baseline serum bilirubin level has also been evaluated in predicting toxicity or efficacy among patients receiving irinotecan for metastatic colorectal cancer. Although modest elevations in bilirubin are associated with increased grade 3 to 4 neutropenia in patients treated with irinotecan, the baseline serum bilirubin is not reported to reliably predict overall irinotecan-related toxicity or efficacy.80 Additional methods, including DNA sequencing and fragment analysis, have been compared with the Invader assay. All 3 methods were valuable for genotyping the UGT1A1 (TA)n repeat, with the sequencing and size-based assays found to have the fewest drawbacks.81

As with tamoxifen, UGT1A1 genotyping is not routinely performed when predicting irinotecan toxicity in current clinical practice. There are many appropriate explanations for the low usage of this test.27 For example, although genotyping has been consistently associated with the hematologic toxicity induced by higher doses of irinotecan, the risk is reduced at the lower doses used in combination therapy.6 Another concern is the potentially decreased efficacy if the irinotecan dose is prospectively reduced because, to our knowledge, no studies have been performed to date to demonstrate that efficacy is preserved in this context. In addition, the lack of
established reimbursement, and the lack of education for clinicians regarding the potential value (and limitations) of testing have limited the utility of UGT1A1 genotyping. Nevertheless, the discovery, validation of candidate genetic variants, and establishment of useful genotyping methods in predicting irinotecan-related toxicity was a paradigmatic success in pharmacogenetic research and translational studies.

5-FU

5-FU has been widely used in the treatment of solid tumors (eg, colorectal cancer) and remains the backbone of many combination chemotherapy regimens. Despite its clinical benefit, 5-FU is associated with frequent gastrointestinal and hematologic toxicities, which often leads to treatment discontinuation. 5-FU undergoes complex anabolic and catabolic biotransformation. Gene products involved in this transformation include dihydropyrimidine dehydrogenase (DPD or DPYD), which breaks down 5-FU, and those drug targets (eg, thymidylate synthase [TS or TYMS]) and methylenetetrahydrofolate reductase (MTHFR). Several of these genes have been shown to affect 5-FU treatment outcomes.

Both genetic and nongenetic factors have been associated with 5-FU toxicity. Watters et al used LCLs from white pedigrees to demonstrate that approximately 26% to 65% of human variations in susceptibility to 5-FU-induced cytotoxicity are due to genetic components. In addition, both age and sex have been shown to influence 5-FU clearance and female sex is reportedly associated with more frequent and severe 5-FU toxicity.

Candidate gene approaches have demonstrated associations between 5-FU treatment outcomes and germine polymorphisms in DPYD, TYMS, and MTHFR. DPYD, encoded by DPYD, plays a key role in the catabolic breakdown of 5-FU. The DPYD gene is located in human chromosome 1p22 and is comprised of 23 exons encompassing approximately 950 kilobase pairs. To date, greater than 30 SNPs and deletion mutations have been identified within DPYD, although the majority of these variants have no functional consequences on enzymatic activity. The intronic variant IVS14 + 1 G > A (DPYD*2A, rs3918290), which has been found in up to 40% to 50% of patients who developed grade 4 neutropenia, has been associated with DPD deficiency. The frequency of low DPD enzymatic activity has also been shown to vary significantly among different ethnic subpopulations, with higher mean DPD activity reported in Korean subjects and lower activity reported in African Americans when compared with white individuals.

TYMS is the primary target of 5-FU. A tandem repeat polymorphism in the 5'-untranslated region (UTR) and a 6-base pair insertion/deletion polymorphism in the 3'-UTR of TYMS have been reported to be associated with altered TYMS expression and clinical response. In addition, MTHFR is a key enzyme that forms the reduced folate cofactor essential for TYMS inhibition by 5-FU. Two linked nonsynonymous SNPs, 677C>T and 1298A>C, have been shown to alter enzyme activity and possibly 5-FU sensitivity.

More recently, a prospective study conducted by Schwab et al evaluated all these potential genetic predictors of 5-FU treatment-related toxicity. They found the DPYD*2A allele to be a risk factor for severe mucositis and leukopenia in men but not in women, and the TYMS 5'-UTR tandem repeat to be associated with diarrhea; however, the clinical utility of these polymorphisms is limited due to poor sensitivity and specificity. The authors suggested that future approaches using genome-wide association to identify additional candidate genes are warranted.

Several clinical assays have been developed assessing DPD activity, mRNA expression, and metabolite formation, as well as SNPs within DPYD. In addition to issues related to sample preparation, additional equipment requirements, and time consumption, one main issue preventing the clinical application of these assays is their limited sensitivity and specificity. For example, Morel et al have shown that the sensitivity, specificity, and positive and negative predictive values of the detection of the 3 major SNPs (IVS14 + 1 G>A, 2846A>T, and 1679T>G) in DPYD as factors predictive of 5-FU toxicity were 0.31, 0.98, and 0.62 and 0.94, respectively. They found that only 50% to 60% of patients who carry genetic variations in DPYD develop severe 5-FU toxicity. This was recently confirmed by Schwab et al. Furthermore, a less pronounced genetic contribution of TYMS polymorphism has been demonstrated in a larger prospective study conducted in 683 patients, in whom the TYMS 2/2 genotype was found to increase the risk of toxicity 1.56-fold compared with the findings of Lecomte et al.
in a study of 90 patients, in whom the *TYMS* 2/2 genotype was found to have a grade 3 or 4 toxicity rate of 43% whereas only 3% of patients who had the *TYMS* 3/3 genotype developed those toxicities.\textsuperscript{101} Recently, Myriad Genetics, Inc. has marketed the TheraGuide 5-FU test to predict toxicity to 5-FU (and capecitabine). This test consists of sequencing the *DPYD* gene, the identification of known mutations (eg, IVS14 + 1 G>A, D949V, and I560S), and polymerase chain reaction amplification of *TYMS* 5' - UTR tandem repeats. The test was designed based on several clinical studies\textsuperscript{100,103} and was reported to have high technical specificity and sensitivity (<1% false-positive variant call and <1% false-negative call; available at: http://www.myriadtests.com/provider/doc/TheraGuide-5-FU-Technical-Specifications.pdf; Accessed November 20, 2008). However, to our knowledge, this test has not yet been approved by the US FDA and its clinical utility requires further study, as exemplified by the recent study by Schwab et al.\textsuperscript{82}

**Conclusions**

We have proposed a five-stage architecture for PGx research. With the exception of a few drugs/markers, the majority of current PGx research efforts are still focusing on the second and third stages (screening/identifying and validating genetic markers). The field of PGx has made enormous strides in recent years with rapid advances in the fields of systems biology and genomics, which provide PGx researchers with many new tools with which to investigate the genetic markers for drug response and toxicity. High-throughput technology has allowed the complete sequencing of the genomes of 2 individuals: Dr. James Watson\textsuperscript{104} and Dr. J.C. Venter.\textsuperscript{105} A simplified version of human genomic interrogation has been launched by a commercial company.\textsuperscript{29} Unfortunately, there is an imbalance between the rapid development of genotyping technology, the marketing of genetic tests, and the uncertainty in interpreting the clinical significance. Except for the few examples listed in this review, we lack PGx for common chemotherapeutics such as alkylating agents. To move the field forward, the clinical utility and economic impact of PGx are in need of evaluation, encompassing the education of prescribers and payers as well as further research providing guidance on alternative treatments or dosages.

PGx has great relevance in cancer therapy because cytotoxic chemotherapeutic agents are, in general, nonspecific with a narrow therapeutic index, often resulting in serious or even fatal toxicities. In addition, most agents are only effective in a subset of patients receiving the drug.\textsuperscript{106} Many exciting new PGx markers have been identified in both germline and cancer DNA through either candidate gene or genome-wide approaches. The techniques are well-established for the candidate gene approach, and the cost is considerably less when compared with the genome-wide approach. There is a greater rationale for studying the genetic variants that arise from a candidate gene study in a clinical setting. However, because response to chemotherapy is likely to be multigenic, the genome-wide approach will be more likely to identify subtle changes in several genes conferring sensitivity to a drug, and may even identify a single polymorphism with a large effect, such as was recently demonstrated for statin-induced myopathy.\textsuperscript{107} For the clinician, the outcome of these studies will be similar to those of any new investigative technology. The first results will need to be verified in similar populations by independent groups. Then, the usefulness of the variants for clinical practice will depend on their improving diagnostic prediction or fostering changes in prevention or treatment strategies. There is a compelling need for more, and more efficient, epidemiologic studies so that these new approaches can be exploited. To meet this need, scientists and clinicians must collect information, informed consent, and tissue samples in the expectation of future studies that will address potential future questions.\textsuperscript{108}

On May 21, 2008, President Bush signed the Genetic Information Nondiscrimination Act (GINA), which protects Americans against discrimination based on their genetic information, into law. The long-awaited measure, which was debated in Congress for 13 years, will pave the way for people to take full advantage of the promise of personalized medicine without fear of discrimination (available at: http://www.genome.gov/24519851; Accessed November 20, 2008). The Genomics and Personalized Medicine Act was introduced by Senator Barack Obama to overcome the scientific barriers, adverse market pressures, and regulatory obstacles that have stood in the way of better medicine\textsuperscript{109} (available at: http://www.govtrack.us/congress/bill.xpd?bill=s109-3822;
Accessed November 20, 2008). The US FDA has also taken the initiative in aiming to facilitate the integration of PGx into drug development and clinical practice.\textsuperscript{110}

In summary, although there are still questions to be answered, PGx researchers now have improved tools with which to take cancer treatment to the next level. With increasing numbers of novel PGx markers being identified and validated, oncologists will have a new means with which to make treatment decisions for their patients to maximize benefit and minimize toxicity for each patient based on the genetic composition of the individual.\textsuperscript{111}

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