Guidelines and Recommendations for Laboratory Analysis and Application of Pharmacogenetics to Clinical Practice

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Guidelines and Recommendations for Laboratory Analysis and Application of Pharmacogenetics to Clinical Practice
(National Academy of Clinical Biochemistry)

OUTLINE

Abstract

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ABSTRACT

Objective: The objective of this LMPG on pharmacogenetics (PGx) is to provide a systematic rigorous assessment of the discipline of pharmacogenetics as it applies to clinical laboratory testing and its application to clinical practice. Issues to be addressed will be: methodological (pre-analytical and analytical) considerations, standardization and quality assurance of testing; selection of appropriate PGx testing profiles; recommended reporting of test results and interpretation; standards needed for demonstration of clinical utility and efficacy; and, regulatory and other recommendations for effective use of pharmacogenetic information in a clinical setting.

Background. Although still in its infancy, the availability and application of information derived from pharmacogenetic-related clinical laboratory tests has provided major in-roads and expectations to the concept of personalized therapeutics. PGx as a clinical adjunct to selection and dosing of drugs is relatively new and as such, medical practitioners, clinical laboratories, in-vitro diagnostic manufacturers and regulators of diagnostic tests have not established evidence-based guidelines needed to optimize practice in their respective areas of application. These present Guidelines provide a framework on which to build a rigorous and systematic approach to establishing optimum use of pharmacogenetic-information obtained from clinical laboratory testing. The Guidelines also establish criteria and critical pathways that must be met before the efficacy of this testing can be rigorously assessed.

Approach: An expert committee drafted evidence-based recommendations (where available) pertaining to the areas of focus. An external panel of experts will review a draft of the Guidelines which will be modified in response to reviewer's suggestions. A revised draft will be posted on the NACB website (www.nacb.org). As guidelines are being developed, parts will also be presented for discussion and awareness at various professional meetings. A working draft will be presented for open discussion at the AACC 2006 Annual Meeting (Chicago, IL). Recommendations will again be modified after input from these activities and subsequently submitted for publication in CCJ (or /and other) before end of 2006.

Summary: We recognize that this relatively new application derived from combining genetic–testing with traditional pharmacology is rapidly evolving and as such the guidelines are likely to also evolve rapidly. Nevertheless, these present guidelines will serve as a basis on which to establish a rigorous approach to defining the applications of this discipline to clinical practice and to define critical pathways and provide the laboratory support needed to bring this application to routine healthcare.

PREFACE

The field of clinical pharmacogenetics is evolving very rapidly. Thus, the Academy has taken the position that establishing preliminary guidelines to help guide the entry of this discipline into the practice of laboratory medicine would be of value to clinical laboratories, healthcare practitioners and regulators, as well as manufacturers of products that are currently shaping the landscape of this important clinical application.

In general, the approach our Committee used to prepare these guidelines for the analysis of and clinical application of pharmacogenetics information was to establish a series of questions pertaining to each of the sections outlined below. The **questions** are listed at the beginning of each section and then a series of **recommendations** are linked to each of the questions posed. Each of the sections (chapters) was originally prepared by the primary authors listed (red) and subsequently reviewed for content by the indicated reviewers to date (blue) for each of the sections.

I. GENERAL INTRODUCTION AND SCOPE

(Author: R. Valdes)

A. Objectives of the LMPG for pharmacogenetics

The Objective of this document is to establish practice guidelines for application of pharmacogenetics (PGx) in the practice of laboratory medicine.

This document will be used to develop an understanding of the present evidence supporting the application of pharmacogenetics-testing to general clinical practice and specific areas of medical practice. We will:

1) Define requirements for (a) adequate and (b) optimal pharmacogenetics-testing in specific clinical settings. Examples include turn-around time requirements for test results, number of alleles needed on test reports and advisability or need for interpretative reporting.

2) Define the potential links in the roles of pharmacogenetics and therapeutic drug monitoring in clinical settings

3) Discuss and formulate recommended guidelines for clinical laboratories introducing pharmacogenetics-testing services

4) Provide in-vitro diagnostic companies guidance on clinical assays and their performance characteristics in pharmacogenetics-testing. Which tests are needed, with what analysis times, etc.?

5) Provide third party payers and regulators of diagnostic laboratory testing recommendations for optimizing their reimbursement and regulatory functions

B. Approach to Grading the Evidence (In Preparation)

THIS SECTION ON GRADING THE EVIDENCE IS IN PROGRESS
(Will be linked to our specific document)

Regarding the evidence-base for this particular set of guidelines involving pharmacogenetics, it is noteworthy that recent published studies have shown poor correlations between the methodological quality used in preparing guidelines in general and the validity of recommendations provided (Clin Chem 2006;52:1,65-72). Thus, in disciplines which are rapidly evolving (e.g., pharmacogenetics) and where the evidence is as yet uncertain, the need exists for documents offering the opportunity to include a series of balanced thoughts behind the recommendations and to also identify gaps in the knowledge and include different options when the evidence is unclear or lacking. Thus, robust recommendations can be forwarded even without a more desirable rigorous evidence-base approach typical of more well–established applications. In fact, as recently indicated by JS burgers (Clin Chem 2006;52:3-4), guidelines are particularly needed in disciplines with areas

of uncertainty compared to more established applications as may be readily available in textbooks.

The GRADE approach has been recommended to be applied when possible to determine the quality and strength of evidence that will be used for a practice guidelines. The approach uses two tables to compile and compare the data from various studies. The first table should have the following columns: List of Studies, Design, Quality, Consistency, Directness, Sparse Data (y or n), Strong association (yes or no), Reporting bias (yes or no), and plausible confounders (yes or no). Where appropriate, other key indicators can be added to the column to facilitate analysis such as dose response, confidence interval, etc. The studies should be listed under each row that defines a particular outcome that is deemed important for the guidelines. A second table summarizes the findings with columns describing the outcome, key genotypes or questions, the effect which can be subdivided into confidence interval and absolute values, the quality and importance of each outcome. Evidence can be graded as follows: highest quality evidence derives from randomized trials, moderate quality evidence is quasi-randomized, low evidence is observational studies, and very low evidence are other studies. The study quality, directness, association and the effect of plausible confounders further define the quality of the evidence. Examples of table formats are listed below is used as a basis for grading the evidence in recommendations in this set of guidelines (although the discipline is still young) and recommended to be used in future more detailed preparation of guidelines in diagnostic pharmacogenetics.

BMC Health Services Research 2005, 5:25 doi:10.1186/1472-6963-5-25

Appendix B. Strength of Recommendations
(Modified from US Preventive Services Task Force Recommendations for Preventive Services)

A. The NACB strongly recommends adoption; there is good evidence that it improves important health outcomes and concludes that benefits substantially outweigh harms.

B. The NACB recommends adoption; there is at least fair evidence that it improves important health outcomes and concludes that benefits outweigh harms.

C. The NACB recommends against adoption; there is evidence that it it is ineffective or that harms outweigh benefits.

I. The NACB concludes that the evidence is insufficient to make recommendations; evidence that it is effective is lacking, of poor quality, or conflicting and the balance of benefits and harms cannot be determined.

NACB grades the quality of the overall evidence on a 3-point scale:

I: Evidence includes consistent results from well-designed, well-conducted studies in representative populations.

II: Evidence is sufficient to determine effects, but the strength of the evidence is limited by the number, quality, or consistency of the individual studies; generalizability to routine practice; or indirect nature of the evidence.

III: Evidence is insufficient to assess the effects on health outcomes because of limited number or power of studies, important flaws in their design or conduct, gaps in the chain of evidence, or lack of information.

C. General scope of pharmacogenetics (PGx) as a discipline in laboratory medicine

Pharmacogenomics and, more specifically pharmacogenetics, deals with the use of information derived from analysis of gene variations for purposes of guiding the use of medications and related therapeutics. Typically, this discipline has two functional component arms that link pharmacology to genetics. These arms are best divided into predicting how drugs are processed by the body (pharmacokinetics) and how they interact with receptors to cause an anticipated response (pharmacodynamics). Typically, pharmacokinetics is strongly linked to biotransformation of drugs by metabolic processes (e.g., by the liver) and their subsequent elimination by kidney function. In contrast, pharmacodynamics deals with understanding the interaction of drugs with receptors and the subsequent response, albeit some biotransformation may be involved. Thus, the former concept as related to PGx-testing in laboratory medicine is usually associated with predicting the biotransformation of a drug by detecting specific genetic variants that control the production of enzymes responsible for biotransformation (e.g., CYP450 enzymes) or transport of drugs (e.g., MDR1). Most of the work done to date, and thus testing available to clinical labs, has focused on this element of PGx. However, genetic variants linked to receptors have indeed been studied and represent a powerful direction in predicting response to pharmacological therapeutics (e.g., Her2nue). Throughout these present guidelines we will attempt to distinguish between these two modalities, but in general, we focus primarily on the pharmacokinetics because more activity has recently surfaced in that application.

Several compelling forces presently exist that drive the need for guidelines such as those presented in his document:

- Rapid flux of knowledge (guidance needed)
- Evidence for clinical application not clear
- Education needed (required)
- Technology transitioning (research to IVD & clinical applications)
- Payers and Regulators confused
- Clinical laboratories (central to transition)

What role might the clinical laboratory play in moving the clinical application of PGx forward? The optimum role of the clinical laboratory relative to pharmacogenetics might be summarized as follows:

- Develop genetic profiling strategies to maximize sensitivity and specificity of predicting phenotype
- Develop methods to reduce testing cost and technical difficulty
- Provide availability of testing
- Overcome barriers to pharmacogenetic genotyping

In context with the above, the following guidelines have been developed for the purpose of this document serving as a guide towards the future development of pharmacogenetics as a discipline in laboratory medicine.

II. PHARMACOLOGY AND POPULATION GENETICS CONSIDERATIONS AND THEIR APPLICATIONS IN PHARMACOGENETICS

(Authors: G. Ruano / R.Valdes; Reviewers: Wendell W Weber and Jose De Leon)

A. General Introduction. Objective of this section is to give the reader a primer in the principals of drug metabolism and population genetics with sufficient basis for understanding how the concepts of genetics are applied in the development and application of pharmacogenetic testing as a discipline.

Questions for consideration are:

a) What are the essential elements of drug pharmacokinetics and pharmacodynamics necessary to understand the application of pharmacogenetics in laboratory medicine?

b) What is the cytochrome P450 system and what are the relevant allele frequencies of these components (CYP2D6, CYP2C19, and CYP2C9)

c) What are key considerations and recommendations for statistically sampling of the indicated alleles in populations

RECOMMENDATIONS

a) What are the essential elements of drug pharmacokinetics and pharmacodynamics necessary to understand the application of pharmacogenetics in laboratory medicine?

B. Introduction to drug metabolism, pharmacokinetics and pharmacodynamics

The half-lives of drugs in blood. The use of therapeutic drug monitoring TDM (or "management" as it is now often referred) has revealed wide differences in the plasma half-life of commonly used medications (see Table taken from Weber 1999). In addition, multi-modal distributions of drug concentrations in blood of patients ingesting comparable amounts of drug have been reported (Weber 1999). Genetic variants associated with drug-metabolizing proteins have been implicated as the basis for these observations, particularly as it pertains to variation in the processing of drugs (pharmacokinetics variation) by individuals.

Person-to-person variation in plasma elimination half-life

DRUG	Half-life variation	Fold variation
Dicumarol	7 – 74 hrs	10
Isoniazid	0.5 – 7.5 hrs	>10
Nortriptyline	15 – 90 hrs	6
Phenytoin	10 – 42 hrs	4
Tolbutamide	4 – 10 hrs	2.5
Warfarin	15 – 70 hrs	>4

Receptors and the concentration of drugs in blood. The theory of "receptor occupancy" is central to understanding drug-action and is linked to the effective concentrations of a drug in blood. The relationship between the concentration of a drug in blood and the response caused by that drug is shown in Figure 1 (Panels A and B) for two conditions. Panel A depicts the dependence on the number of receptors available and panel B the affinity of the

receptors. Both situations might be affected by genetically-based contributions, for example: a genetic variant in the promoter region may reduce the number of receptors or a genetic variant in the coding region may affect the affinity of the receptors. A second important concept to understand is the relationship between the dose of a drug ingested and the concentration ("drug burden") of the drug in blood. In simple terms, the descriptive elements that dictate the concentration-time curve or area under the curve (AUC) of a drug in blood are shown in Figure 2 (Panels left and right) including the subsequent action on the target cells (taken from Weber 1999) and hence link to the importance of receptor mediated responses.

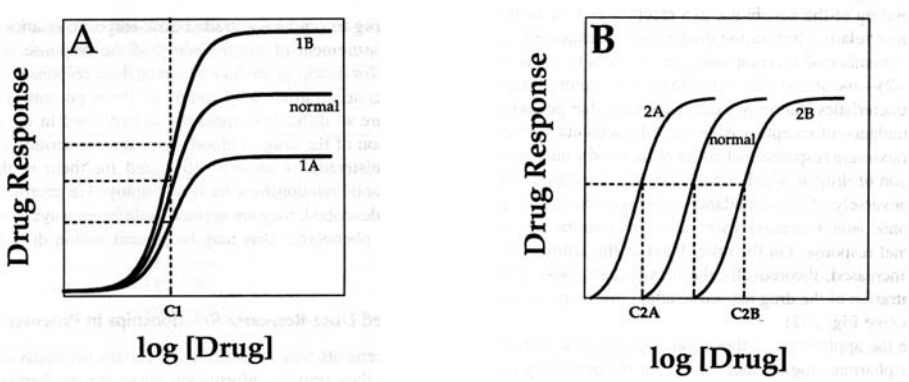

Figure 1. Panel A shows relationship between drug response and number of receptors where 1A is low number and 1B is high number of receptors. Panel B shows the relationship of drug response to affinity of receptors, where 2A is a high affinity receptors and 2B is a low affinity. Note that the 50% effective drug concentration C2A is lower for a high affinity receptor compared with concentration C2B.
(Taken with permission from Weber 1999)

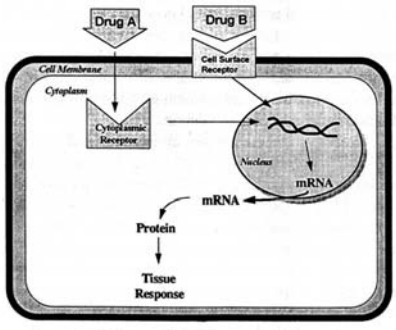

Figure 2–1. The profile of human drug response.

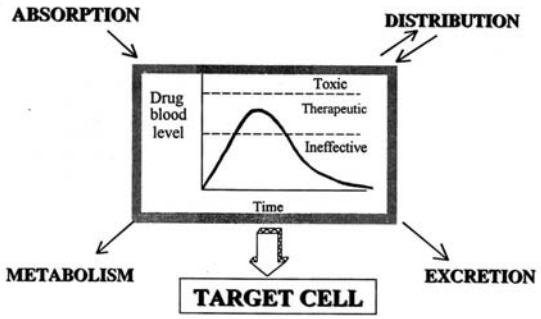

Figure 2. Left panel: shows the area under the curve (AUC) for a drug dose and the elements controlling the shape and magnitude of the curve, typically: absorption, distribution, excretion and metabolism. Right panel: shows the subsequent interaction of the drug with a cell surface receptor or a cytoplasmic receptor. The affinity and number of the receptors are linked to discussion in Figure 1 above. (Taken from Weber 1999)

Liver as a drug-metabolizing organ. Although other organ systems are known to metabolize medications, the liver is by far the principal organ responsible for processing and providing major pathways for the biotransformation of many ingested medications. The concentration-time curve shown above is controlled by a combination of drug bioavailability, biotransformation (metabolism), and subsequent elimination by the kidney.

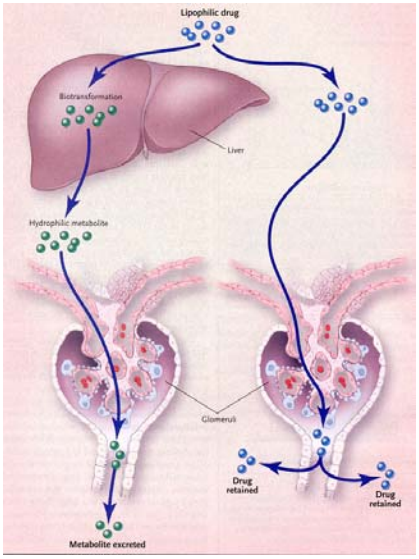

Figure 3. Lipophilic drugs are biotransformed in the liver for subsequent excretion by the kidney. Without biotransformation a lipophilic compound is typically reabsorbed. (Taken from: Weinshilboum NEJM 2003;348:529-537 (review)

Genotyping and drug dosing requirements. On a practical basis, one might ask if there is evidence that the concentration-time curve in response to drug dosing is affected by genotype linked to a pharmacogenetic variant (i.e., test result). The figure below taken from a recent review by Brockmoller (2000) shows a relationship between the area under the curve (AUC) and the genotype for CYP2C19 relative to the drug omeprazole. It serves as an example of the marked effects that a genetic variant may have on the important dynamics of drug metabolism and accumulation in blood. Note that a homozygote CYP2C19*2/*2 has an AUC almost 9 time higher than a *1/*1 homozygote (wild type, i.e., normal genotype). Additionally, a heterozygote also has a reduced elimination of this drug manifested by a 2-fold higher AUC compared to normals.

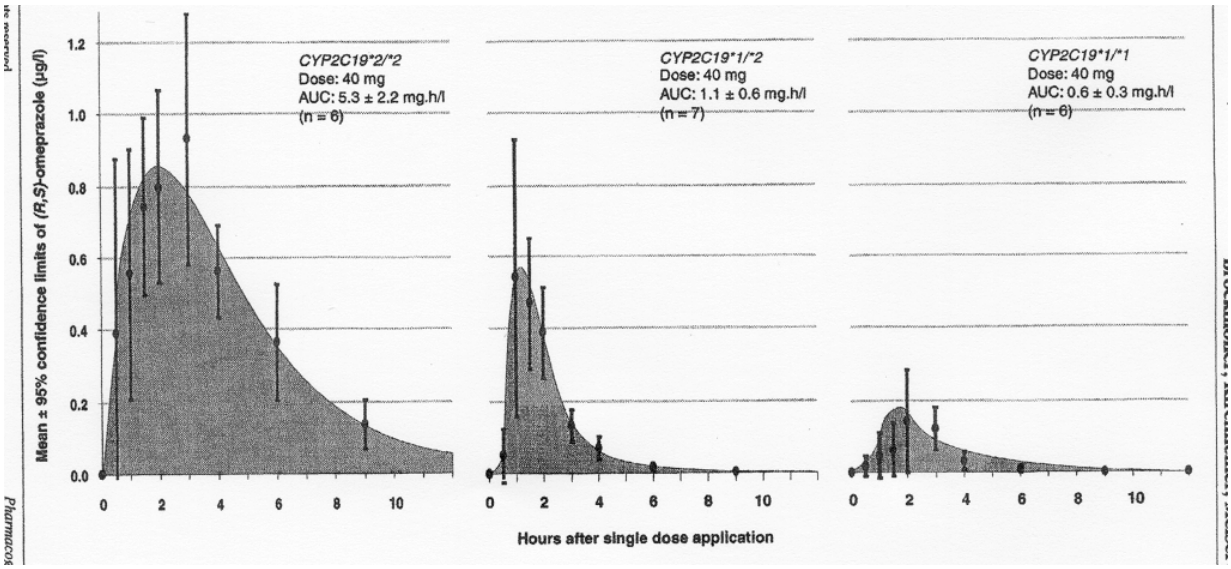

Figure 4. Taken from Brockmoller J, et al. Pharmacogenomics 2000;1:125-152 (review)

Adverse drug reactions (ADR). Systematic studies involving review of published literature indicate that adverse drug reactions are indeed prevalent and associated with costly hospitalizations (JAMA). Such studies indicate that over 2.2 million such events are frequently observed in the USA, and that a substantial fraction (>100,000 per year) involve subsequent mortality. In addition, published literature reviews show a link between ADRs, classes of drugs, and genetic variants of key metabolizing enzymes. One review suggests that 59% of the drugs associated with reported ADRs are metabolized by liver enzymes for which genetic variants have been described, compared to only 20% for the randomly selected drugs.

From the perspective of this LMPG, it may be important to suggest that the definition of adverse drug reaction (ADR) be expanded to include other situations that can lead to costly medical intervention including complications due to or leading to the loss of drug efficacy, reduced compliance, the misclassifications of physician ordering patterns, psychological implications to patients, and other complications leading to reduced healthcare efficiency as well as increased costs. Many of these situations are known to occur, but are not now classified as important as an ADR which may require immediate hospitalization, or causing morbidity or mortality. Nevertheless, these less well-defined conditions do lead to costly and unnecessary medical interventions. This later suggestion may be of practical importance due to the advancing age of worldwide populations, the increased number of individuals taking multiple drugs (polypharmacy, see figure below), and the ever-increasing cost of healthcare. Another influential factor is the now predicted use of disease-preventive therapies base on prophylactic use of medicinal agents which may not have immediate biological markers as surrogates for efficacy. Thus, achieving and maintaining appropriate concentrations of drug in blood would be essential.

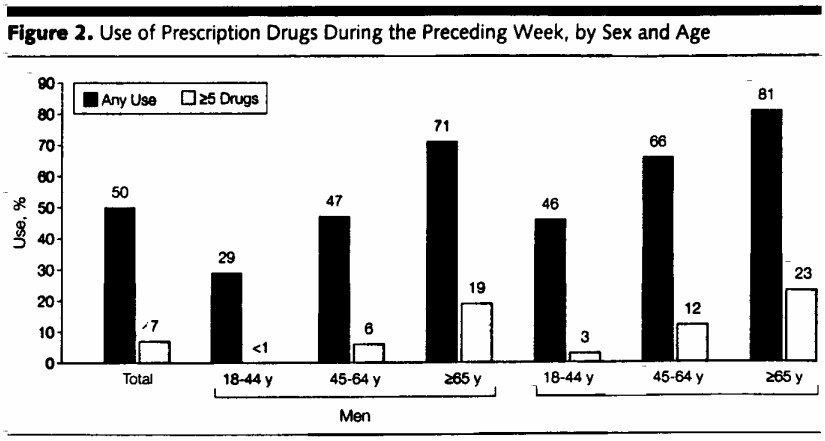

Figure 5. This graph shows the percent of individuals by gender and age who are taking prescribed medications. Note that for individuals over 65 years of age, upwards of 70% are using more than 5 medications (i.e., polypharmacy). (Taken from: Kaufman et al., JAMA 2002;287:337-344)

In any event, substantial documentation exists to address the efficacy of major drugs used to treat several important diseases and their dependence on a pharmacogenetic component (see Spear et al, Trends in Molec Med 2001:7(5):201-204).

b) What is the cytochrome P450 system and what are the relevant allele frequencies of these components (CYP2D6, CYP2C19, and CYP2C9)

C. Introduction to the cytochrome P450 system

Cytochrome P450 (CYP) is a family of heme-containing enzymes that catalyze the conversion of lipophilic substances into hydrophilic molecules which can then be excreted by the kidneys into the urine. It represents a major part of the body's powerful detoxification systems. The CYP system metabolizes endogenous and exogenous substrates through a variety of reactions including epoxidation, N-dealkylation, O-dealkylation, S-oxidation and hydroxylation. Exogenous substances (products ingested or absorbed) include not only pharmaceutical compounds given as therapeutic drugs, but also foodstuffs and dietary components, and occupational pollutants and industrial chemicals. The CYP mixed-function monooxygenase system is probably the most important element of Phase I metabolism in mammals. More than half of all drugs are primarily cleared by the CYP system (Bertz and Granneman, 1997, deLeon et al 2006, Wilkerson 2005).

The CYP P450 system has evolved into a gene family expanded into multiple chromosome loci, each with tandem arrays of genes, and each gene with substantial polymorphism. This system is an illustration of gene expansion, multi-gene families, and allelic functional variation. Genomics has supplied a rich resource of gene mapping data as well as the individual variants in each gene at the single nucleotide polymorphism (SNP) and chromosome locus levels. The 57 CYP P450 isoforms now known in humans, along with the hundreds of genetic variations, have produced a large set of biomarkers predictive of susceptibility to specific toxins. The fact that the pharmaceutical industry routinely includes an assessment of the main metabolic pathways of a candidate drug to derive clinical pharmacological correlations is indicative of the importance of this knowledge (Ruano et al. 2004).

Metabolization Status and Drug Dosing Recommendations	CYP2C9	CYP2C19	CYP2D6
	Prevalences	Prevalences	Prevalences
Poor metabolizers (PM) are at increased risk of drug-induced side effects due to diminished drug elimination or lack of therapeutic effect resulting from failure to generate the active form of the drug. Individuals have a deficiency in drug metabolism.	**3%**	**4%**	**10%**
Intermediate metabolizers (IM) may require lower than average drug dosages for optimal therapeutic response. In addition, multiple drug therapy should be monitored closely.	**30%**	**38%**	**40%**
Extensive metabolizers (EM) represent the norm for metabolic capacity and therefore posses the full complement of drug metabolizing capacity. Generally, extensive metabolizers can be administered drugs which are substrates of the enzyme following standard dosing practices.	**67%**	**58%**	**49%**
Ultra-extensive metabolizers (UM) have increased metabolic capacity and may require an increased dosage due to higher than normal rates of drug metabolism. Simultaneously treating with medication that inhibits metabolization has also proven effective.	**0%**	**4 to 18%**	**1%**

Table 1. *Metabolizer Status, Drug Dosing Recommendation, and Population of Cytochrome P450 Phenotypes in surveys of individuals of Western European ancestry)*

CYP2D6: The variability of metabolism of debrisoquine and sparteine was discovered in the late 1970s (reviewed by Meyer and Zanger, 1997) in what is now the classic example of phenotypic variability ascribed to isoenzymes, which we now know result from gene polymorphism. The metabolic variability could be traced to the P450 CYP2D6 gene. This gene was found to be hypervariable, which required analysis of multiple single nucleotide polymorphisms (SNPs) as well as of its deletion and duplication in some individuals (Kirchheiner *et al.*, 2001, 2004). In what may be an example of adaptive evolution, hypervariability in this gene is potentially advantageous, which would be consistent with the need to process and detoxify different substances in various environments. Three decades of research in this gene exits and phenotypic correlates to these patterns of variation are now available. At least 70 alleles have been described (www.imm.ki.se/CYPalleles/cyp2d6.htm), and the phenotypic characteristics of their diploid constitutions in humans ascertained (Bertilsson *et al.*, 2002). This variability is typically distilled into four phenotypes (Table 1): ultra-rapid (UM), extreme (EM), intermediate (IM) and poor metabolizers (PM). However, it should be pointed out that there is no agreement on how to define an intermediate metabolizer. One definition is that a person with one active allele and a null allele is an intermediate metabolizer (Kircheiner et al, 2004). The other definition for a CYP2D6 intermediate metabolizer is a subject with a null allele and one with low activity (eg., *10) (Chou WH, et al., 2003). The clinical perspective on this is more closely addressed in the Section below on Clinical Considerations.

Previous studies have demonstrated marked ethnogeographic variance in the distribution of CYP enzymes throughout the world. For the CYP2D6 gene, in particular, the allele frequencies evidence significant diversity, and representation of alleles known to have multi-continent segregation. The non-European common alleles most likely observed in a USA urban population are alleles *17, *10 and the gene duplication. Allele *17 is reported to be found predominantly in individuals of African ancestry. Allele *10 is found predominantly in individuals of Asian origin. The gene duplication allele is found predominantly in individuals of origin traceable to Northern Africa and the Middle East (McLellan et al., 1997; Droll et al 1998). Results for major urban centers may show the inherent diversity of populations in the service area, and to the recognition of DNA typing as a major tool in dealing with the diversity of our patients. In any event, it should be noted that 20-25% of all drugs are subject to metabolism by CYP2D6, and that while more than 70 CYP2D6 variants have been identified, several laboratories have reported that most poor metabolizers can detected by screening for up to five alleles (Daly et al., 1996).

The distribution and frequency of these phenotypes is dominated by reliance on "race" for their determination, which may not be suitable for the modern practice of pharmacogenetics. Published estimates routinely offered point to a 10% frequency of the PM phenotype in individuals of Western European ancestry ("Caucasians") (where the CYP2D6*4 allele is common) (Droll *et al.*, 1998), and a frequency of only 1% in individuals of Far East Asian ancestry (Rossi, 2004). Intermediate metabolizers account for 40% of the "Caucasian" population. The extensive metabolizer phenotype is the most common in Caucasian populations, accounting for half the population. Differences in response among populations of different ethnogeographic origin can often provide clues to the influence of genetic factors on individual susceptibility to the efficacy and toxicity of specific drugs. Perhaps the best-known example is "primaquine sensitivity" due to G6PD deficiency among African and Mediterranean peoples (Vulliamy et al., 1988). Another important example is the remarkable difference in sensitivity of Caucasians compared to Japanese to the adverse effects of ethanol attributed to ethnic differences in the variant isoforms of the enzymes that metabolize ethanol and acetaldehyde (Shibuya and Yoshida, 1988; Higuchi et al, 1992). Additional examples of ethnogeographic variation associated with CYP2D6 and CYP2C19 are discussed below.

CYP 2C9: A host of drugs including Fluoxetine, sertraline, warfarin, acenocoumaral, tolbutamide, losartan, phenytoin, and glipizide are metabolized by this enzyme. The most common polymorphisms are *2 (Arg-144-Cys, C-430-T SNP and *3 (Ile-359-Leu, A-1075-C SNP). Both are functionally deficient alleles studies (review by Xie et al, 2002). Both *2 and *3 show reduced catalytic activity (increased Km) and /or decreased maximum rate of metabolism (decreased Vmax) *in vitro*). The CYP2C9 gene is of great relevance to cardiovascular medicine as it metabolizes warfarin, a standard treatment for thromboembolism. For *in vivo* correlations, *3 heterozygotes and homozygotes were demonstrated to have reduced (S)-warfarin clearance by 66% and 90%, respectively. Also, *2 or *3 carriers require lower maintenance dose of warfarin, and experience a first bleeding event sooner with standard unadjusted dosages, consistent with a poor or intermediate

metabolizer phenotype. Finally, *3 homozygotes are poor metabolizers for warfarin, acenocoumaral, tolbutamide, losartan, phenytoin, and glipizide.

CYP2C19: The CYP2C19 polymorphism was first identified as S-mephenytoin hydroxylation polymorphism (Kupfer and Preisig, 1984). Diverse pharmacologically important therapeutic agents, such as antidepressants (*i.e.*, citalopram, tricyclic), anti-epileptics and proton pump inhibitors have been identified as substrates. Several variant alleles of CYP2C19 cause the expression of inactive enzyme. The mani variants are allele *2 to *8, but other more rare variants from *4 to *8 have been described. Hence heterozygosity for any of this alleles leads to a poor metabolizer phenotype and requires drug dosage adjustment (Kirchheiner *et al.*, 2001, 2004). Similar to CYP2D6, the variability in CYP2C19 allele frequencies varies significantly between ethnicities. The poor metabolizer status is found at a frequency of 2-5% in Caucasian and of 13-23% in Asiatic populations. As indicated in Table above, recent studies indicate that 2C19 is also associated with rapid metabolizer phenotypes (Sims et al., 2006) the frequency of which was population-dependent (18% in Swedish and Ethiopian populations and 4% in Chinese).

c) What are key considerations and recommendations for statistically sampling of the indicated alleles in populations

Recommendation 1: The genotype and frequency of alleles from the genes offered for pharmacogenetic tests should be determined in the service area where the data will be utilized. The survey should include carrier status of the population.

Rationale. Determining the frequency and diversity of polymorphisms in a typical clinical population will serve to raise the awareness of variability in functional status of patients in the service population as a justification for individualized drug therapy.

The survey should include the alleles of most clinical significance for each gene based on the molecular nature of the polymorphism and the frequency of the allele. Examples using polymorphisms in CYP2D6, 2C9 and 2C19 are in the previous section above.

Recommendation 2: The distribution of alleles in a service population should be evaluated with regard to a reference population to assess service-specific enrichment of alleles. We recommend a survey of 100 to 150 individuals in the service population.

Rationale. Comparison of the average number of functional and deficient or null alleles in the service population with that in a reference population is required to ascertain a service-specific allele database for any segregation enrichment. The samples typed in the survey can be compared against published frequencies for the alleles in various populations such as in the Table above. For the purposes of assigning a quantitative phenotype, ultra metabolizers are carriers of more than two normal alleles, extreme metabolizers, carriers of two normal alleles, intermediate metabolizers, carriers of 1 normal and 1 deficient or null allele, and poor

metabolizers, double carriers of deficient or null alleles. The sample size needed to detect a deviation of the mean number of functional alleles is given by

$$n = \frac{\sigma^2 (Z_{1-\alpha/2} + Z_{1-\beta})^2}{\Delta^2},$$

where σ is the standard deviation (assumed equal between the two samples), Δ is the difference between the means to be detected, α = 0.05 is the significance level, and 1-β = 0.8 or 0.9 is the desired power. The Figure shows the dependence of the detectable Δ on sample size, showing that at the recommended 100-150 samples a difference of 0.25-0.30 alleles is well within the range of detection. The two most likely reasons for a difference include an ethnogeographic ancestry distinctive to the service population and referral bias of patients with deficient and null alleles to tertiary care centers for medical management complications. For either of these reasons or both, it is desirable to publish these regional and service-specific surveys. Each center will have specific features pertinent to the population it serves which are of great value for the entire field of pharmacogenetics as a whole and to the development of the new standard of care based on individualized therapy. For a general discussion on statistical considerations relevant to pharmacogenetics, please refer to Holford et al 2005.

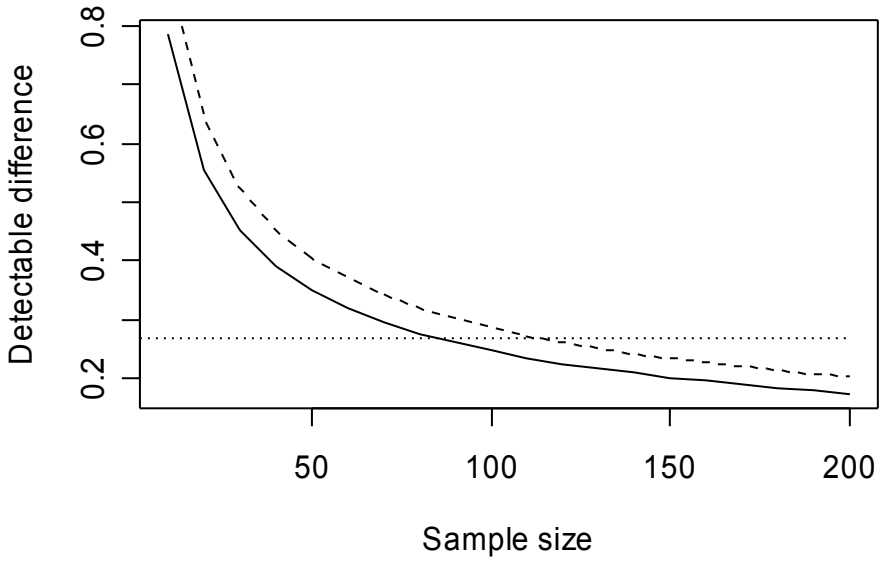

Figure 6: Difference in mean number of functional alleles that can be detected vs. the number of patients genotyped. For power of 80% (solid line), and 90% (dashed line). The dotted line is between 0.25 and 0.30 difference.

Classical population statistics such as Hardy Weinberg equilibrium may have limited applicability to a population sample of diverse ethnogeographic ancestry typical of urban populations.

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III. Methodology and quality assurance considerations in Pharmacogenetic testing

(Author: D. Payne; Reviewer: Jeanne Carr)

General Introduction. When performing Pharmacogenomics testing, the laboratory should follow the CAP guidelines for Molecular Pathology testing. Because molecular diagnostic testing has been present in many laboratories for approximately 10 years, many quality assurance and quality control issues have been identified and addressed through those guidelines. With this said, the technology used for determining genotype is becoming more complex and will require additional measures to assure reliable and acceptable test results. The goal of the present PGx guidelines is to facilitate reproducible testing intra- and inter-laboratory pharmacogenomic testing that is independent of test platform.

Questions for Consideration

a) What is the error rate for each test and each platform?

b) What potential haplotypes, single nucleotide polymorphisms, pseudogenes, epigenetic modifications, or GC ratios could produce inaccurate results for each instrument and/or assay?

c) What substances or specimen types could interfere with the various instrument platforms?

d) Do laboratory methods correlate closely with clinical manifestations? What roles do genotypes versus phenotype assays have? What are the roles of each of those assays? What artifacts can make the assays produce conflicting data?

e) What reference/control material will be used for validation, proficiency, and lot-to-lot Quality control? How much data is needed for adequate validation? How often is proficiency, analyst competency, and QC to be performed?

f) If software is used in interpretation, will it automatically flag extremely rare or unlikely allele combinations?

Recommendations

a) What is the error rate for each test and each platform? *Example: Some platforms may not be able to detect large repeats and thus may report a patient as being homozygous wildtype versus heterozygous, or some primer sets may erroneously amplify one allele versus another.*

Recommendation 1: There should be evidence that discrepant results from various instrument platforms or within the same instrument platform have been investigated and corrective action taken. Sequencing analysis as the gold standard for variant detection should include sequence data from both strands. Other methods may be

considered the Gold standard for some variants such as Flourescent in situ hybridization (FISH)

Rationale 1: Some platforms may not be able to detect large repeats and thus may report a patient as being homozygous wildtype versus heterozygous. FISH may be able to detect large deletions or duplications but not small nucleotide changes. Sequencing may not detect a large deletion of one allele but can identify single nucleotide changes.

Rationale 2: Statistics of patient population frequencies must be maintained in the laboratory to detect any potential errors in testing. These population frequencies must be obtained from the patient test population and not cited from nonregional published data. CAP Molecular Pathology Checklist November 2005

b) What potential haplotypes, single nucleotide polymorphisms, pseudogenes, epigenetic modifications, GC ratios could produce inaccurate results for each instrument and/or assay?

Recommendation 2: The error rate for each pharmacogenomic test must be determined for each platform that is used in the laboratory. This should be conducted by analyzing pooled DNA samples from a renewable well characterized resource such as Corriell cells. The limit of sensitivity must be at least 10% variant in a background of 90% wildtype.

http://ccr.coriell.org/nigms/products/snp500.
http://ccr.coriell.org/nigms/dna/dnapools.html or
http://ccr.coriell.org/nigms/cells/humdiv.html
www.gentris.com

Rationale: Some methods preferentially detect one variant or allele versus another variant. Detection of 10% admixture of variant assures that the platform is functioning properly. (feedback per AMP membership at Annual Meeting (November 2005), Beutler E et al Blood Cells Mol Dis 2000, Jeffrey G, et al Nat Genet. 1999, Chen B et al Nature Genetics (In Press)

c) What substances or specimen types could interfere with the various instrument platforms?

Recommendation 3: Controls that detect potential inhibitors for assays that utilize enzymes should be incorporated into the assay. These controls may be an exogenous target or the assay should be designed so that the restriction fragment analysis produces cleaved product for both wildtype and variant targets.

Rationale 1: Extracted nucleic acids are less likely than crude samples, such as specimens from fecal or plasma nucleic acids, to contain inhibitors toward enzymatic amplification or target detection, i.e. restriction fragment length analysis or flap enzymatic analysis. Controls that detect potential inhibitors for assays that use enzymes, such as allele specific

amplification, restriction enzyme digestion or cleavase based assays should be incorporated into the assay on each run (CAP Molecular Pathology Checklist November 2005)

d) Do laboratory methods correlate closely with clinical manifestations? What roles do genotypes versus phenotyping assays have? What artifacts can make them produce conflicting data?

Recommendations 4: There should be evidence that molecular test results are correlated with non nucleic acid based clinical tests when possible.

Rationale 1**:** Correlation of molecular and non nucleic acid assays provides quality control and assurance for the test. Performing such correlations will permit the identification of new variants or other factors that may result in erroneous results. These correlations can utilize literature references, in house clinical outcome data, case reports or in house inter-assay data. (Feedback Per membership at annual AMP meeting (November 2005), CAP Molecular Pathology Checklist November 2005)

e) What reference/control material will be used for validation, proficiency, and lot-to-lot Quality control? How much data is needed for adequate validation? How often is proficiency, analyst competency, and QC to be performed?

Recommendation 5: Assay validation should include whole genomic samples from both genders and the ethnic groups that will be tested by the assay when possible. Additional testing should contain whole genomic samples possessing homozygote wildtype, heterozygote, and homozygote variant when possible. The validation should include adequate numbers of specimen types that will ultimately be tested. For example, if the test is to be performed on buccal swabs, whole blood and paraffin sections, then validation studies must be performed to determine the performance characteristics on each of these sample types. Proficiency testing should be performed twice annually.

Rationale 1. Ideally, proficiency should include whole genomic specimens that have been tested by another laboratory for the particular assay. In the case where no such proficiency samples are available, previously tested samples may be de-identified and a key to the de-identified samples retained. The key must not be released to the testing personnel until completion of the proficiency run. Whole genomic amplification of rare variants is permitted subsequent to validating the performance or quality of this material against non-amplified materials. This recommendation recognizes that extremely rare variants may not be amenable to this recommendation due to the scarcity of samples. In this case, the assay can be validated using a Gold standard assay to initially validate the genotype. Upon validation, the sample can be used within the laboratory in an alternative proficiency testing biannually. CAP Molecular Pathology Checklist November 2005

Super controls that detect all possible variants may be used for confirming lot-to-lot differences and daily quality assurance. Synthetic DNA controls may be used in this case. Alternatively, a subset of pooled specimens may be used to check the lot or daily runs in numerous runs. The QA for each variant should be performed in a timely fashion to permit

retesting if that assay fails to pass appropriate quality assurance. Beutler E et al Blood Cells Mol Dis 2000, Jeffrey G, et al Nat Genet. 1999, Chen B et al Nature Genetics (In Press)

Rationale 2: Ethnicity affects allelic frequency. CAP Molecular Pathology Checklist November 2005

Rationale 3: Amplification potential differs for various sources of nucleic acids. Buccal swab samples may contain less nucleic acid than blood samples. Paraffin samples have size constraints for amplicons compared to whole blood samples thus preventing the use of long range PCR analysis for haplotyping.

Rationale 4: Proficiency testing is required to assure and improve the quality of laboratory testing. CAP Molecular Pathology Checklist November 2005

Rationale 5: The quality of whole genomic amplification depends on the quality of the source nucleic acids. Poor quality reference DNA can result in non-identical allelic representation for the whole genomic amplified samples. Therefore, the ability of the whole genomic amplified sample to function as a control must be determined prior to its incorporation into the assay. Chen B et al Nature Genetics (In Press)

Rationale 6: The use of controls to monitor and/or confirm test performance between lots and from assay to assay are required to identify failed assays in a timely manner. Timely identification of failed runs allows for corrective action and avoid delaying patient results. CAP Molecular Pathology Checklist November 2005

f) If software is used in interpretation, will it automatically flag extremely rare or unlikely allele combinations?

Recommendations 6: Software should use either a consensus-expert opinion rule-based logic system and/or a relational database. If possible, the software should include clinical cutoffs for relevant genetic variants.

Rationale 1: When possible, software logic should be implemented that will discern alerts or detect unlikely genetic results for either a combination of genetic variants or an ethnic group which might signal a QA issue with the assay. If possible, pharmacogenomic data should be easily retrieved or viewed on the patient's electronic medical record (EMR) when the presence of genetic variants associated with life threatening adverse drug reactions have been identified (i.e. EPIC EMR possess a header with patient name and known allergies and Mycobacterium tuberculosis (Mtb) results). 1997-2005 Epic Systems Corporation

Rationale 2: Interpretation between assay results may vary from one laboratory to another. Forming a consensus group that defines interpretations will avoid presenting the patient with one result from one laboratory versus another result from a different laboratory.

De Luca A et al Antivir Ther. 2004, Gallego O et al J Virol Methods. 2004, Mcleod HL et al Nature Genetics 2005.

Rationale 3: Software logic permits the laboratory to identify potential faulty results if the test generates a combination of genotypes from a patient that is statistically impossible or improbable. This tool will permit the laboratory to repeat the assay prior to reporting the result to the patient, thus assuring test quality and patient safety.

Recommendation 7: Whenever possible, laboratories should design and validate their testing system and protocols to address the necessity for duplicate collection, testing and reflexive testing.

Rationale 1: Some assay platforms may lack appropriate sensitivity and specificity for particular alleles. In these situations, reflexive or repeat testing may improve the analytical performance of the assay. Example, some assay platforms may fail to detect large deletions resulting in erroneous homozygous results from the nonvariant allele.

Rationale 2: The identification of extremely rare variants may require a second collected sample and assay for confirmatory purposes.

Rationale 3: Pharmacogenetic testing can be viewed as a test result that would not require repeat testing. Thus an incorrect result due to specimen mix-up could potentially impact a patient on multiple occasions throughout their lifetime. Therefore a recommendation for duplicate specimen collection and testing could be viewed as a prudent measure. However this recommendation would double the cost of performing such testing. Thus a well designed testing system that is validated to mitigate the risk of specimen mix-up is recommended. Laboratories should document and challenge their testing systems for their ability to avoid transposition of test results between individuals. An example of such a system would be to repeat testing when a genotype is not consistent with the ethnicity of the patient

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IV. CLINICAL LABORATORY SERVICES CONSIDERATIONS

(Authors: M. Linder and W. Steimer; Reviewers: Dennis O'Kane, Elaine Lyons)

General introduction. Providing clinical laboratory services is of central importance in establishing appropriate utilization of pharmacogenetic information to clinical practice. Thus, the laboratory must provide services consistent with the needs of healthcare providers. To provide these services in the context of pharmacogenetic-testing it is essential that, as with other clinical testing domains, laboratory operations and procedures be rigorously controlled. Because PGx is in its infancy it becomes important to utilize not only well-established and rigorous laboratory practice, but to also consider processes that may be necessary to ensure adequate compliance that may be either unique or somewhat specific to the development of this new discipline combining pharmacology and genetics in the context of laboratory medicine.

Questions for consideration:

a) What level of certification should be required for clinical laboratories and personnel performing pharmacogenetics testing?

b) What are the recommended specimens for testing?

c) What should be the primary test-result output?

d) What test result turn around times are optimum for PGx testing?

e) What criteria should be used to establish which genetic variants of a locus should be included for diagnostics purposes?

f) Is it necessary for there to be evidence to demonstrate cost effectiveness before recommending clinical use of PGx tests?

RECOMMENDATIONS

a. What level of certification should be required for clinical laboratories and personnel performing pharmacogenetics testing?

Recommendation 1: Whenever possible, and when required for laboratory compliance with local regulations, personnel performing pharmacogenetic testing must be qualified to perform "high complexity testing" as defined by CLIA Subpart A Sec 493.17(a). Further it is recommended that personnel performing pharmacogenetic testing have one to two years experience in molecular diagnostic techniques (CLIA Subpart M Sec 493.1489) and be also trained in the basic principles of therapeutic drug monitoring/management.

Rationale 1: Three categories of laboratory services have been established including waived, moderate and high complexity. A determination of testing complexity is made based on a cumulative score for seven criteria each ranked as 1, 2 or 3, with 1 representing little complexity and 3 representing the highest complexity. The following table represents the scoring based on a consensus of the authors of this document.

Criteria	Rank
Scientific Knowledge	3
Training and experience	3
Reagents and materials preparation	2 - 3
Characteristics of operational steps	2-3
Calibration, QC, and proficiency testing materials	3
Test system troubleshooting and equipment maintenance	2 - 3
Interpretation and judgement	3
Cumulative score	18 - 21

Tests having a cumulative score of > 12 are considered to be are considered to be of high complexity (CLIA regulations subpart 493.17).

Rationale 2. An understanding of basic pharmacological principles is essential for personnel performing and supervising pharmacogenetic testing. Examples are presented in sections VI and VII of this document.

b) What are the recommended specimens for testing?

Recommendation 2: Whenever possible it is recommended that robust diagnostic methods be developed and optimized to analyze genomic DNA obtained from fresh whole blood, dried whole blood spots, isolated nucleated blood cells, oral epithelial cells obtained from either buccal scraping or saliva.

Rationale 1: Service requirements, technical capabilities and healthcare provider safety issues are increasing the demand for molecular diagnostics that do not require genomic DNA isolated from blood. The availability of techniques that can reliably use non-blood sources of genomic DNA avoid barriers to testing and enable collection of the specimen at the point of care when on site phlebotomy is not available. Due to variability in DNA quality obtained from alternative sources, assay reliability for all intended sources must be validated.

c) What should be the primary test-result output?

Recommendation 4: Laboratories should report a description of ALL physical characteristics of the genetic locus that is being determined by the assay.

Rationale: Despite efforts to standardize nomenclature systems for genetic variation, sufficient ambiguity remains and newly discovered features of previously defined alleles continue to antiquate their earlier designation. Further, not all assays for a given locus will include the same features. Thus it is recommended that all of the features tested (defined as objective findings CAP MOLPATH checklist MOL. 36000) of the genetic locus be specifically identified. As an example, in lieu of reporting CYP2C9 *1/ CYP2C9 *2 the laboratory could report: CYP2C9 430C/T (heterozygous). Alternatively the former nomenclature can be used however the physical feature(s) used to define that allele should be clearly stated on the report. The remaining genotype would be CYP2C9 430C/C for homozygous active, and CYP2C9 430T/T homozygous reduced. Both an accepted nomenclature and commonly used designations should be used on reports. The committee is cautious about recommending a specific format at this time. This is being discussed for other inherited conditions, and a consensus is not yet reached.

d) What test result turnaround times are optimum for PGx testing?

Recommendation 5: Laboratories should provide turn-around times that are consistent with the clinical application of pharmacogenetic test results. In general, the goal is for the physician to be advised of the patients genotype in sufficient time to avoid risk to the patient.

Rationale: To support drug selection and dosage, assay results should be available such that any risk to the patient is avoided. There are no published studies to demonstrate at what point in time for an individual drug and situation, the pharmacogenetic result will be required. For explanation of failure of response or adverse reaction and for the purpose of general medical future knowledge of a subject's PGx- genotype the results are not particularly time sensitive.

In contrast, if PGx testing is applied in a screening mode results should be available prior to or co-incident with the application of therapy or no longer than the time anticipated for drug concentrations to accumulate beyond what would be the expected concentrations in extensive metabolizers. Review of the warfarin accumulation models shown in section VI for example would suggest that pharmacogenetic test results should be available prior to the fourth dose to avoid overdosing of patients with CYP2C9 variants.

Recommendation 6: Using PGx tests for purposes of targeted screening should be restricted to high risk drugs (narrow therapeutic window). A direct or indirect significant correlation with clinical outcome should be evident at least in retrospective studies. In this regard, and regarding polymorphism in drug targets, three independent studies should demonstrate a significant correlation between polymorphism and clinical outcome.

Rationale: Post hoc testing after therapeutic problems have appeared can explain an unexpected course of therapy and provide a rationale for change of therapy. It may also help to avoid unnecessary extra diagnostic procedures and rule out suspected compliance problems. Post hoc testing is usually not particularly time critical. (Note: NCCLS recommends three studies to demonstrate clinical outcome. However, recommendation does not specifically address PGx)

Predicting clinical response and adverse drug reactions of a particular therapy in a particular patient will provide the maximum benefit from genotyping. Such screening will require results to be available prior to initiation of therapy or at least before undesired effects will surface. This may be technically difficult and expensive but severely sick patients seek immediate help and the optimal drug should be chosen prior to initiation of therapy because repeated change is unwanted (e.g. in psychiatry - might interfere with therapeutic success).

General testing of every potential drug prior to therapy carries a prohibitive cost. Testing should therefore be restricted to high risk drugs only. Regarding drug metabolizing enzymes, high risk drugs may be stipulated as those displaying a narrow therapeutic window. Ideally, direct evidence showing a correlation between polymorphism and clinical outcome (e.g. amitriptyline/nortriptyline; Steimer et al. 2005) should be available for a particular drug or at least a drug class. If this is not available, indirect evidence showing a correlation between polymorphisms and drug concentration and on the other hand drug concentration and clinical outcome might be available for some drugs. Case studies and trend reports are not sufficient to propose pre-therapeutic genotyping. As a minimum, retrospective studies reporting significant associations are necessary to be followed later on by confirmatory prospective studies. Because of low prevalences of patients with extreme genotypes (e.g.

CYP2D6 PMs and UMs) and the consecutive difficulties in achieving results with the required level of certainty, it might be beneficial to study populations with a higher prevalence (e.g. IMs and EMs) utilizing more refined tools for genotype/phenotype relationships (gene dose, allele specific change of concentration etc.).

Because conflicting results are extremely common in association studies on drug target polymorphisms and clinical outcome at least two confirmatory studies are required before such tests may be considered.

Recommendation 7: Unless other measures of clinical outcomes are clearly defined, when establishing the predictive value of a PGx test result based on a genotype linked to drug-metabolism, measuring drug burden (area under the drug-concentrations vs time curve) or steady state concentrations in chronic dosing are the recommended "first–approximation" method for assessing predictive value (PV). (Also in previous section)

Rationale: High analytical sensitivity and specificity regarding the detection of a particular wild type or variant allele are generally easily achieved in molecular diagnostic procedures. However, the clinical sensitivity and specificity of pharmacogenetic test results are not as easily defined. The critical component for this assessment is the clinical endpoint being determined. For the validation of such tests, unique biological or pharmacological endpoints are optimal but they are not always currently available for many treatment conditions. More importantly, it will be the association between genotype and clinical outcome that will determine the use and potential value of a genotyping assay. While a variety of different clinical outcomes are assessable, in pharmacogenetics these are typically identified as, for example, adverse drug reactions, non-response to a drug, etc..

In the context of the two examples used through out this document an example is warranted. The pharmacodynamic end point for warfarin therapy is the international normalize ratio (INR), a measure of anti-coagulation. Cytochrome P450 2C9 genotype has been directly associated with increased risk of above range INR's when patients are administered standard dosages and further associated with lower maintenance dosages in stabilized patients. Likewise genetic variants of the Vitamin K epoxide reductase complex 1 protein have been associated with lower maintenance dosages in stabilized patients. These are both good examples of the genetic variants being directly associated with a clinical end point.

Alternatively in the case of atomoxetine and cytochrome P450 2D6, there is a clear association between an increase in the area under the plasma concentration versus time curve and the CYP2D6 PM genotype. The association between CYP2D6 and adverse events is documented, however the specificity of the relationship between these adverse events and the CYP2D6 genotype would limit their usefulness for phenotype-genotype associations.

e) What criteria should be used to establish which genetic variants of a locus should be included for diagnostics purposes?

Recommendation 8A: Whenever possible, PGx assays designed for clinical application should include all known genetic alterations of the target locus for which there is a well-defined influence on the function of the locus product or for which there is a clear relationship between the structural characteristic and observable influence on drug pharmacokinetics, pharmacodynamics and/or toxicology. Also, reference should be made as to dependency on population or allele frequency. For example, a rare variant described once in the literature should not be included in the panel.

For example: In the case of CF, the recommendation is to include all disease causing alleles that have a frequency of > 0.1%. (0.001)

Rationale: The key to this recommendation is the provision that there be a well-defined influence of that feature on a well defined PK or PD endpoint.

Using CYP2D6 as an example the *3, *4, *5 and *6 alleles are reported to account for 98% of inactive alleles in Western European ancestry (or Caucasian) populations. Approximately 7 % of the population are genotypic PM's thus the total frequency of PM alleles must be 0.265. Given that *3, * 4, * 5 and *6 collectively account for 98 % of PM alleles, the cumulative frequency of the remaining alleles is ~ 0.006. Laboratories testing for only the most common four alleles will miss-identify 1 in ~ 22 PM's as heterozygous for one correctly identified PM allele and one allele miss-identified as an active allele. Thus the overall error would be 1 in 300 subjects tested. This is an example only and should be determined for each practice setting taking into account differences in allele distributions arising from differences in geographic genetic exchange. Laboratories should disclose what genetic features are included in their analysis and anticipated sensitivity and specificity of the techniques for discrimination of relevant phenotypes.

Recommendation 8B: Regarding establishing criteria for inclusion of genetic variants in PGx diagnostic assays, the following is recommended:

1. **The variant should be directly linked to a change in function or abundance of the gene product and/or be directly linked to a measurable clinical outcome.**

2. **The cumulative frequency of the variants included in the diagnostic assay should account for > 99% of the variants known to account for the phenotypic end point.**

f) Is it necessary for there to be evidence to demonstrate cost effectiveness before recommending clinical use of PGx tests?

Recommendation 9: Clinical pharmacogenetic tests should have a compelling rationale for their use. Criteria should be established to demonstrate this.

Rationale 1. Compelling reasons for diagnostic assays or procedures include a) a test that mitigates risk of adverse events or b) a test that improves response to therapy or c) a test that reduces the overall cost of treatment.

Rationale 2. Pharmacogenetic testing should be requested at the time of therapeutic intervention and is not currently recommended for general population screening.
The FDA has recently acknowledged the importance of testing for CYP2C9 and VKOR C1 genetic variation during the early phase of warfarin therapy. Further the FDA has acknowledged the importance of testing for TPMT, of individuals to be treated with azathioprine. In addition UGT 1A1 genotype is acknowledged for patients to be treated with Irinotecan. These decisions were largely driven by the strong evidence to suggest that individuals who have these genetic variants are at significantly (statistical term) increased risk of an adverse event when administered these medications. These acknowledgements were not based directly on a cost-effectiveness or QOLY determination.

Recommendation 10. It is recommended that a series of questions be developed and sanctioned to be used as guidelines when assessing the cost-effectiveness of a pharmacogenomic-based treatment strategy as applied to a pre-drug use screening modality.

Rationale 1. Before **pre-drug use** PGx testing can be generally advocated it will first be necessary to demonstrate not only functional effects but associations between genetic variation and clinical outcomes based on adequate populations studies. Proof of cost-effectiveness will likely be necessary to drive the implementation of screening and such studies should attempt to measure outcomes in healthcare quality or cost per event avoided rather than concentrating on intermediate markers.

Rationale 2. Before a pharmacogenetic testing be considered for purposes of cost-effectiveness as applied to general screening, a series of important questions should be addressed. Some examples are:

- What is the frequency of the genetic polymorphism?
- How closely is the polymorphism linked to a consistent phenotypic drug response?
- Are there metabolic, environmental or other significant influences on drug response?
- What are the sensitivity and specificity of the genomic test?
- What alternative tests are available to predict drug response?
- How prevalent is the genotype of interest?
- Is the genotype or haplotype important – does the test detect genotype or haplotype?
- What are the characteristic outcomes associated with the genotype with and without it knowledge?
- How does the pharmacogenomic strategy alter these outcomes?
- What is the therapeutic range of the drug involved?
- What alternative therapeutic options are available?
- How effective are current monitoring strategies for preventing severe ADRs and predicting drug response?

	Factors to assess	Features that favor cost effectiveness
Gene	Prevalence	Variant allele is relatively common
	Penetrance	Gene penetrance is high
Test	Sensitivity, specificity and cost	High specificity and sensitivity
		A rapid and relatively inexpensive assay is available
		No alternative test to individualize therapy is available
Disease	Prevalence	High disease prevalence in the population
	Outcomes and economic impacts	High untreated mortality
		Significant impact on quality of life (QOL)
		High costs of disease management using conventional methods
Treatment	Outcomes and economic impacts	Reduction in adverse effects that significantly impact QOL or survival
		Significant improvement in QOL or survival due to differential treatment effects
		Monitoring of drug response is currently not practiced or difficult
		No, or limited, incremental cost of treatment with pharmacogenomic strategy

It is likely that PGx applications will probably be most cost efficient for drugs:

- With a narrow therapeutic index
- High variability in inter-individual response
- Problems in monitoring ADR or treatment response
- Few alternative treatment options

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V. Reporting and interpretation of pharmacogenetic test results

(Authors: J.P. Morello and R. Valdes; Reviewers: J. Kirchheiner and D. Reynolds)

General Introduction. When reporting genotype information from pharmacogenetic tests, the clinical laboratories must keep in mind that the end user of this information will most likely be the physician or other healthcare provider. It is usually the physician who will report the findings to the patient and take action using the test result. For the physician to correctly interpret the genotype information, it is beneficial that he or she have the complete diagnostic setting of the patient including present and past drug regimens, medical history and lifestyle. The following guidelines will detail several key recommendations and elements that should be included in the clinical laboratory and interpretive report.

Questions for consideration are:

a) What information should accompany the reported result?

b) Should the result be linked to a specific drug usage (as indicator)? Should drug "dosing and usage" information accompany the test result?

c) Should laboratories reporting PGx test results have a genetic counseling component or service available or by referral?

d) Should manufacturers of IVD used for providing PGx tests be required to supply evidence of specific use for every drug, or by class?

e) Are there unique or specific limitations to be considered regarding confidential reporting of PGx test results?

Recommendations

a) What information should accompany the reported result?

Recommendation 1: Laboratories reporting pharmacogenetic genotype test-results should be prepared to provide an educational resource to recipients of the test results to explain the complexity of the metabolic pathways involved and also be prepared to provide guidance as to which genes should be tested for a given clinical situation when that is known.

Rationale: Understanding drug metabolism pathways is central to interpretation of PGx tests. Thus, an appropriate consultation component is essential for optimum clinical application of the test results. Whether the physician is concerned about the effects of a drug he/she has previously prescribed and failed to offer clinical benefit (retrospective analysis) or is concerned about future benefit (prospective analysis), he/she will need to know the contribution of different CYPs to drug metabolism in order to correctly evaluate which CYP gene will require genetic testing. For example, the commonly prescribed antidepressant

citalopram is metabolized to N-desmethylacitalopram by CYP2C19 (Kobayashi *et al*., (1997) Exp. Ther. 280, 927-933; Herrlin *et al*., (2003) Br. J. Pharmacol. 56, 415-421). N-desmethylacitalopram is then metabolized, but by CYP2D6 now, to N-didesmethylcitalopram (Sindrup *et al*., (2003) Ther. Drug Monit. 15:11-17; Olesen & Linnet, (1999) Pharmacology 59:298-309; von Moltke *et al*., (1999) Biol. Psychiatry. 46:839-849). For citalopram, the antidepressant activity resides in the parent compound therefore, even though CYP2D6 plays a role in the overall metabolism of citalopram, it is CYP2C19 which will require genetic testing.

b) Should the result be linked to a specific drug usage (as indicator)? Should drug "dosing and usage" information accompany the test result?

Recommendation 2: Whenever possible and to provide optimum interpretive guidance relative to a specific drug or similar family of drug substrates, a laboratory providing PGx test results should seek information on drug substrates involved in the clinical situation.

Rationale 1: **Associating genotype to clinical metabolizer status.** Without scientifically-validated data, it is dangerous to assign a phenotype to a genotype. This was very well illustrated for CYP2C9 where variant alleles (*2 and *3) are associated with diminished enzyme activity. Studies by Kirchheiner and colleagues have shown that although individuals genotyped as either CYP2C9 *2/*2 or *3/*3 are classified as "poor metabolizers", reduction of drug clearance was highly variable for many drugs (Kirchheiner & Brockmoller (2005) Clin. Pharmacol. & Ther. 77:1-16). This illustrates that overall phenotypic classification will dependent on both genotype and drug (substrate). As we will see in the next section, drug-gene interactions also affect clinical phenotype.

Rationale 2: **Providing patient-specific drug-gene interaction information.** Given the expanding knowledge of drug/CYP interactions, an interpretive genotype report should optimally analyze the patient's current (and past) drug regimen to evaluate the risk of drug/CYP interactions. As an alternative, the report should include at least a few examples of drug/CYP interactions that would caution the physician to consider these types of inhibiting interactions. For example, it is known that a number of antidepressants that function as selective serotonin reuptake inhibitors (SSRIs) can inhibit one or more CYPs and that this inhibition can be anywhere from mild to potent (for a review, see Spina *et al.*, (2003) Fundam. Clin. Pharmacol. 17:517-538). Therefore, even though patients are genotyped as extensive metabolizers for a given CYP, if they are/were taking drugs that have CYP-inhibitory activity, their actual enzyme activity can be as low as that found in poor metabolizers (Jeppesen et al., (1996) Eur. J. Clin. Pharmacol. 51: 73-78; Kohler *et al*., (1997) Pharmacogenetics 7:453-61; Ozdemir *et al.*, (1998) J. Clin. Psychopharmacol. 18:198-207; Alfaro *et al.*, (1999) J. Clin. Psychopharmacol. 19:155-163; Alfaro *et al.*, (2000) J. Clin. Pharmacol. 40:58-66; Lohmann *et al*., (2001) Pharmacol.57:289-295). Once the patient is no longer taking drugs that act as CYP inhibitors, his/her metabolizer status will gradually return to that of an extensive metabolizer.

Recommendation 3: The interpretative PGx genotype report should include a summary section in which the physician or other healthcare provider can have quick access to the key issues regarding the patient's metabolic status and its effect on the drug regime being considered. This section should include, but is not limited to, the following information:

1. **The exact alleles detected and their functional significance, i.e., active or inactive. The nomenclature has been addressed in recommendation #4 of section IV.**
2. **For an interpretative PGx genotype report to be effective, the patient's current drug regimen should be indicated.**
3. **A table indicating genotype-specific drug metabolism information should provide "adjustment considerations" (A/C) for the physician. For example, for a CYP2D6 poor metabolizer on codeine, the A/C statement could be: "Consider using an alternative analgesic" with appropriate explanation as to the reasons be provided.**
4. **A table indicating drug-drug interactions with a statement explaining the nature of the interaction.**

Rationale: This kind of information will make the report more user-friendly and can help gain wider acceptance among physicians. Including information in the test report addressing linking the tests result to drug regimen being considered, frequency of genotype in various ethnic populations, and geographical information needed to assess specificity, sensitivity, and predictive value associated with the end point of the therapy being sought are necessary for appropriate interpretation of the report. For example, the reason a 2D6 PM should not take codeine is different than the reason why a PM should take a lower dose (or an alternate med all together) of morphine. Activation versus metabolism is an important distinction.

c) Should laboratories reporting PGx test results have a genetic counseling component or service available or by referral?

Recommendation 4: Laboratories not equipped to provide test-interpretation capabilities as part of their test-reporting process should seek affiliations with organizations providing those interpretive services in order to generate a more complete test report.

Rationale 1: These Guidelines recognize that interpretive genotyping reports are complex and involve an integration of a number of factors that extend beyond the patient's genotype. Seeking the advice of organization offering these kinds of interpretive genotype reporting can serve as bridge between clinical laboratories and physicians.

Rationale 2: For interpretive genotype reporting to be successful, it must not only give information on problematic drugs in the patient's current drug regimen but should also include a prospective assessment of medications at the physician's disposal. In patients that are poor metabolizers for a given CYP, this list will alert the physician to be cautious with drugs in the same therapeutic area whose metabolism depends mostly or exclusively on that

CYP or be able to administer drugs that are metabolized through a deficient enzyme system at lower than standard dosages. Dose modifications as an option are important, especially when the drug in question has no equivalent substitute.

d) Should manufacturers of IVD used for providing PGx tests be required to supply evidence of specific use for every drug, or by class?

Recommendation 5: A report documenting a PGx test result should provide the recipient of the information sufficient detail documenting the analytical methodology used to obtain the result and address known limitations that may influence the robustness, interpretation, sensitivity and specificity of the test result.

Rationale: The robustness and limitation of a PGx genotyping test results may be limited by the method used to perform the analysis. Ability to detect duplication of alleles, assess their number, be influenced by collection methods, etc., can all play a role in the robustness of the result thus place limitations on the interpretation (Kirchheiner *et al.*, (2004) Clin. Pharmacol. & Ther. 76:302-12; Schaeffeler *et al.*, (2003) Hum. Mutat. 22:476-85). For example, the Tag-It kit from Tm Bioscience can detect that a duplicated allele is present but cannot determine in the heterozygous patient which allele is duplicated. The Roche Amplichip CYP450 system and long-range PCR-RFLP techniques (Gaedigk *et. al.*, (2006) Drug Metab. Dispos. 34:563-9) can determine which allele is duplicated in a heterozygous patient. Neither system identifies the number of additional gene copies that exist. Copy number, particularly in a heterozygous patient, can change the phenotype: *1xN/*4 would be an EM if N=2, but a UM if N=3 or more.

e) Are there unique or specific limitations to be considered regarding confidential reporting of PGx test results?

Recommendation 6: A pharmacogenetic test result should be documented consistent with rules pursuant to routine demographics as required for CLIA compliance and in a manner consistent with protection of sensitive genetic information required by HIPAA guidelines (www.hhs.gov/ocr/hipaa/ guidelines/guidanceallsections.pdf).

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VI. CLINICAL PRACTICE CONSIDERATIONS

(Authors: G. Ruano and MW Linder; Reviewers: J DeLeon and D. Flockhart)

General introduction. One of the most challenging aspects of transitioning the science of pharmacogenetics to the bedside is establishing criteria for its clinical application. Although this discipline is in its infancy, there are several examples that serve as fruitful models on which to establish guidelines and set future criteria for clinical implementation. Pharmacogenetic tests have applications in a wide variety of drug-based therapies some of which are more well-established than others. Our approach for these present practice guidelines is to take several key examples and use them as a basis on which to set criteria for documenting future development of these services to medical practice. The term "practice" in this sense has a broader meaning than is usually ascribed. For example, a practitioner may be a physician wanting to use PGx data to determine dosing or drug-selection for a patient. A clinical researcher designing a study to determine the clinical efficacy of using pharmacogenetic information is also a practitioner with a different goal. A clinical laboratorian establishing a pharmacogenetics laboratory is also practicing clinical PGx when deciding on which clinical scenarios to develop testing profiles for maximum clinical impact. Other situations apply as well to the definition of "practice", thus establishing a framework around building clinical practice guidelines for an infant discipline depends on capturing several key examples from both the literature and personal experiences from practitioners. As models, we consider 3 situations in which PGx test results have been reported useful for establishing criteria for clinical applications: *warfarin* (anticoagulation), *atomoxetine* (psychiatry), and *irinotecan* (oncology). These three models combined demonstrate several strategies and concepts for future development of PGx applications.

The metabolic status of the patient revealed by DNA typing should not be considered a disease, but an adverse environmental interaction to an exogenous agent, a drug therapy. The phenotype is triggered by exposure to the agent, but in its absence does not convey overt pathology. Thus, DNA typing for drug safety presents a new capability to diagnose eminently preventable conditions such as drug induced syndromes. As such it should gain more awareness in modern healthcare. The prospective use of DNA typing poses the potential of individualized therapeutic management and and advances personalized medicine. The prospective use of appropriately selected or modified doses of psychiatric and cardiovascular drugs metabolized by the CYP enzymes constitutes the basis for modeling of individualized or personalized medicine.

Questions for consideration.

a) Which are the most current variant alleles for CYP2D6, 2C9, and 2C19 recommended for clinical use?

b) What set of criteria (characteristics) should be required of a PGx test to make it clinically useful?

c) What examples are available that can presently be used as models for application of PGx-testing in clinical settings? warfarin (2C9 and VKOR), atomoxetine (2D6), irinotecan (UGT1A1).

d) What models of PGx-genotyping are available which can serve to establish dosing adjustment rules based on PGx information?

e) What role should the clinical laboratory play in developing the use of PGx testing?

RECOMMENDATIONS

a) Which are the most current variant alleles for CYP2D6, 2C9, and 2C19 recommended for clinical use?

Recommendation 1: The following variant alleles are recommended when performing PGx-genotyping for 2D6, 2C9, 2C19

CYP2D6			CYP2C9			CYP2C19		
Variant	**Alleles**	**Activity**	**Variant**	**Alleles**	**Activity**	**Variant**	**Alleles**	**Activity**
Wild type	***1**	Normal	Wild type	***1**	Normal	Wild type	***1**	Normal
Duplication of *1	**Dupl.**	Ultra	430C>T	***2**	Decr.	681G>A	***2**	*Null*
Deletion	***5**	*Null*	1075A>C	***3**	Decr.	636G>A	***3**	*Null*
-1584C>G	***2A Promoter**	Decr.	1076T>C	***4**	Decr.	1A>G	***4**	*Null*
100C>T	***4, *10**	Decr.	1080C>G	***5**	Decr.	1297C>T	***5**	*Null*
124G>A	***12**	*Null*	818delA	***6**	*Null*	395G>A	***6**	*Null*
883G>C	***11**	*Null*				IVS5+2T>A	***7**	*Null*
1023C>T	***17**	Decr.				358T>C	***8**	*Null*
1707T>del	***6**	*Null*				-806C>T	***17**	Ultra
1758G>T	***8**	Decr.						
1846G>A	***4**	*Null*						
2549A>del	***3**	*Null*						
2613-2615 del AGA	***9**	Decr.						
2850C>T	***2, *17**	Decr.						
2935A>C	***7**	*Null*						

Table. *Alleles of clinical reference for CYP2D6, CYP2C9, and CYP2C19.*

Rationale. The alleles of most clinical significance are precisely those where the molecular nature of the polymorphism has well defined biochemical effects. We can describe the alleles as *null*, *deficient*, *normal*, or *ultra* based on well defined molecular properties of the polymorphism. Null alleles are lacking any biochemical activity at the enzyme level because the gene structure does not produce a functional protein. Such null alleles include gene deletions, frameshift mutations, stop codons, and splicing variations. Deficient alleles have subfunctional enzymatic activity due to a base pair DNA variation that leads to an amino acid substitution in the protein. The range of functionality of the deficient alleles is usually assessed in vitro. Ultra refers to gene duplications which result in a supra-functional level of enzymatic activity. It should be noted that not all duplications are active. Duplications of alleles *1 and *2 are active, whereas duplications of null alleles such as *4 are associated

with no activity. * Methods used in clinical diagnostics should be capable of distinguishing duplication of active versus inactive alleles.

The table above is an example of allele listings annotated with predicted biochemical effects at the protein level. Detection of null alleles leading to compromised metabolizer status is of primary clinical relevance. Additional genetic variation in each of the above enzymes have been reported and are likely to be identified as more individuals are genotyped. Recommendations for inclusion of alleles in diagnostic testing methods will need to be periodically re-evaluated as more data become available. Note: The authenticity of the CYP2C9*4 allele is in question and interpretation of its finding in a clinical sample should be done using extreme caution.

Is should be noted that a particular variant is not always phenotype-specific in that the variant may have a different impact depending on the drug (substrate) in question. For example, the classical literature on CYP2D6*17 suggests that this allele tends to be associated with lower metabolic activity, but the decrease is not homogeneous among substrates. Recent studies using Risperidone (a psychoactive drug) suggests that *17 is associated with at least normal metabolic capacity for Risperidone metabolism (See Cai et al., in press). This suggests that *17 may influence various drugs differently as previously described (Wennerholm et al, 2002). Similar substrate specific effects for CYP2C9 have been documented, for example celecoxib metabolism is not altered in the case of the CYP2C9*2 allele.

b) What set of criteria (characteristics) should be required of a PGx test to make it clinically useful?

Recommendation 2: For a PGx test to be clinically useful it is recommended that it have characteristics similar to other diagnostic tests in clinical use.

Rationale. All diagnostics tests have analytical and operational features that render them useful and compatible with clinical practice. The following are general such characteristics that apply to pharmacogenetic tests as well:

1) *Analytical reliability.* The test should yield consistent measurement of the desired analyte.

2) *Operational implementation.* The test should have operational characteristics within the level of complexity certified by CLIA for reference clinical laboratories.

3) *Clinical predictive power*. The interpretation of the test should have levels of specificity and sensitivity consistent with other diagnostics in clinical use.

4) *Compatibility with therapeutic management.* The interpretation of the test should be useful for guiding therapeutic management and decision making.

c) What examples are available that can presently be used as models for application of PGx-testing in clinical settings? Warfarin (2C9 and VKOR) ; Strattera (2D6); Irinotecan (UGT1A1)

WARFARIN (model)

Recommendation 3: A combination of CYP2C9- and VKOR-genotyping are the recommended PGx tests as adjuncts to individually adjust dosage for Warfarin therapy

Rationale. Warfarin is a frequently prescribed drug for both the treatment and prevention of thromboembolic complications. More than 21 million prescriptions are written annually in this country for warfarin. Warfarin is a narrow therapeutic index medication with frequent complications despite dose adjustment for clinical variables including age, gender, weight, nutritional factors and interactive medications. Such complications range from occult bleeding to hemorrhage. Warfarin is metabolized to inactive metabolites by the Cytochrome P450 2C9 subfamily of drug metabolizing enzymes (Linder et al 2002, Sconce et al 2005). Approximately 25% to 35% of the population have 2C9 alleles that lead to variably deficient enzyme activity and 3 to 4 % of the population have 20% or less 2C9 drug-metabolizing activity. These variants can be detected by DNA analysis. These CYP 2C9 variants account for approximately 25% of the overall variability in Warfarin dose. These variants lead not only to variable initial warfarin dose sensitivity but also to delays in achieving a stable maintenance dose, delays in hospital discharge and increased bleeding complications.

Vitamin K Epoxide Reductase Complex protein 1 (VKORC1) is a recently characterized allelic abnormality in Vitamin K metabolism (Reider et al 2005, Sconce et al 2005). VKORC1 is necessary to reduce oxidized Vitamin K to the reduced form required for post-translational maturation of the Vitamin K-dependent Clotting Factors II, VII, IX and X. Genetic variants increase or decrease the responsiveness of this system and account for an approximate additional 25% of clinical variance in Warfarin dosage. Since the 2C9 and VKORC1 act independently the total genomic based Warfarin variability is presently believed to be at least 50%.

Recommendation 4: A clinical practice model should be developed to provide guidance for use of CYP2C9 and VKORC1 genotyping in conjunction with current standard of care.

Rationale: In November of 2005, the clinical pharmacology advisory panel to the FDA acknowledged the importance and potential for genotyping of CYP2C9 and VKORC1 during the early phase of warfarin therapy. However, most studies document the relationship between CYP2C9 and VKORC1 genotypes and warfarin maintenance dosage and offer little guidance regarding the initial dosage titration period. We have developed pharmacokinetic models based on S-warfarin clearance measured in subjects having variant CYP2C9 alleles. The anticipated consequence of standard therapy in terms of accumulation of plama S-warfarin is modeled in figure 1. This model predicts that

Recommendation 5: The following is recommended as a general dosage adjustment model for Warfarin based on 2C9- and VKOR-genotyping:

a) Begin therapy with standard Warfarin dosing load
b) Establish 2C9 and VKOR genotyping before first dose adjustment (usually 3 to 5 days)
c) Combine PGx results with patient physical attributes (weight, age, etc.)
d) Adjust Warfarin dosing based on recommendations indicated below

Rationale: The CYP2C9 family consists of the normal, wild type, rapid metabolizer 2C9*1/*1, the *2/*2 variant intermediate metabolizer and the *3/*3 poor metabolizer. Additionally compound heterozygotes *1/*2, *1/*3 and 2*/3* occur. The CYP 2C9 variants account for approximately 25% of the overall variability in Warfarin dose. In addition, several studies have demonstrated significant relationships between, S-warfarin plasma concentrations at steady state, warfarin maintenance dose and VKORC1 (-1639G>A) genotype (Sconce 2005, Zhu 2006). Due to their effects on S-warfarin clearance or the amount of S-warfarin required to inhibit the formation of active clotting factors, these variants can lead to delays in achieving a stable maintenance dose, delays in hospital discharge and increased bleeding complications.

There has been little discussion in the literature regarding considerations for induction and transition protocols to apply pharmacogenetics in a prospective fashion. However, several key pieces of evidence are available to support the development of these protocols. Currently, induction of warfarin therapy involves an initial dose of 5 or 10 mg. Subsequently, patients are then treated with the average maintenance dose of □ 5 mg daily, which usually results in an INR □ 2.0 after 4 or 5 days (Hirsh Circulation 2003). Dosages are then adjusted according to the INR. Peyvandi et al reported that during the induction phase of warfarin therapy, warfarin dosages did not significantly differ between subjects with variant CYP2C9 alleles until the 4th to 5th days of therapy (Peyvandi). Further, we have developed pharmacokinetic models of S-warfarin accumulation to steady-state by applying S-warfarin clearance determined for each of the most common CYP2C9 genotypes (Linder et al. J Thrombosis Thrombolysis 2002). Based on differences in the time to reach steady-state plasma concentrations of S-warfarin attributable to the CYP2C9 genotype, rational decisions can be made with regard to the interval between dosage modifications and INR measurements. For example, patients with the CYP2E1 *1/*1 genotype will reach steady-state within 6 to 7 days of dosage modifications, in contrast, interpretation of the relationship between dose and effect on INR should be cautiously interpreted for patients having the CYP2C9*1/*2 or *3 genotypes unless the INR measurements occur 12 to 16 days following dosage modification.

These data support the following prospective approach to application of pharmacogenetic diagnostics to warfarin therapy. This scenario models a three day turn around time for pharmacogenetic diagnostic testing. In our experience, this is a sustainable level of service that can be maintained by the majority of laboratories offering these services

(http://www.pgxlab.com). Patients can be treated based on standard induction protocols (5 mg/d) for the first three to four days of therapy with dose adjustments directed towards minimizing risk of above range INR's. Samples for pharmacogenetic testing should be collected on the first day of therapy and results analyzed in the context of dose estimation models as reported in this and other studies (Sconce). Once the estimated maintenance dose is determined, the clinician will need to make an informed decision regarding subsequent dosing based on the perceived greater risk of thrombosis versus hemorrhage or bleeding. The physician may choose to omit the dose on day 5 when the estimated maintenance dose is << 5 mg and the INR is already near or within the target range, and then commence with the estimated maintenance dose for the time required (e.g. 5 elimination half-lives for the CYP2C9 genotype (Linder et al)) to achieve steady-state. Alternatively when the estimated maintenance dose is >> 5 mg, and the INR is well below the target, the clinician may choose to administer one dose that exceeds the estimated dose and commence with dosing with the estimated dose for the appropriate period to again ensure that INR measurements are interpreted in the context of the time to reach steady state. This approach is anticipated to recognize the majority of variables that influence warfarin dosage titration and yield superior out comes with regard to safety and efficacy. For those patients who are already being treated, this information may assist by again having specific information regarding that individuals receptor sensitivity or S-warfarin clearance which significantly influence the interpretation of INR monitoring. The authors recognize that there may be a transition in the care team responsible for managing patients on warfarin. Thus, anti-coagulation clinics will need to coordinate with referring facilities in terms of ordering diagnostic testing and ensuring that results are reported to the appropriate follow-up care setting.

Alternative recommendations have been made regarding adjustment of Warfarin dosages based on CYP2C9 genotype (Personalized Medicine 2004). These recommendations do not include the effects of the physical characteristics of individual patients nor do they take VKORC1 variation into account.

Warfarin Sodium (Coumadin®)
Dosage Recommendations by Genotype

Genotype	Dosage
*1/*1	100%
*1/*2	87%
*2/*2	82%
*1/*3	68%
***2/*3**	**57% ****
***3/*3**	**33% ****

** Consider alternatives

S-warfarin accumulation models base on genotype-directed dosing

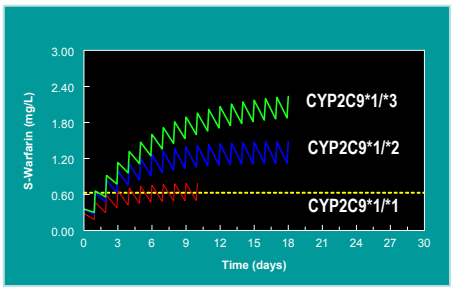

Fig 1. Standard 5 mg dose

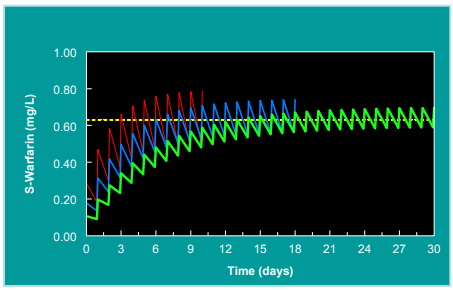

Fig 2. Genotype-specific maintenance dose

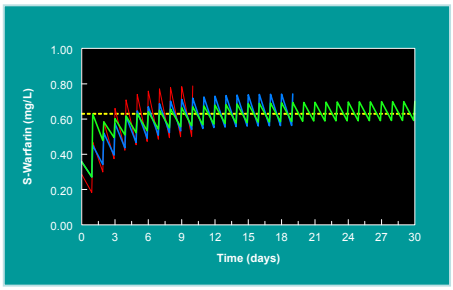

Fig 3. 5 mg initial dosing followed by genotype-specific maintenance dosing

Linder et al. J Thrombosis & Thrombolysis 2002;14:227-232

ATOMOXETINE (Strattera) as a model

Recommendation 5: Genotyping for CYP2D6 should be performed as part of the regimen for prescribing atomoxetine (Strattera). It is also a model for other drug-related dosing applications

Rationale. The close apposition of DNA typing and drug label recommendations and advisories presents an immediate role for personalized medicine in modern healthcare. The product insert label for atomoxetine already has significant CYP2D6 guidelines. The label for atomoxetine, used in Attention Deficit Hyperactivity Disorder (ADHD) in children, adolescents and young adults warns that poor metabolizers have plasma concentrations that are five times greater than observed when extensive metabolizers are administered the same dosage and, have an increase in half life from 5 to 20 hours (refer to figure 3 section VII). The label lists the side effects from effective overdosage and states "Laboratory tests are available to identify CYP2D6 poor metabolizers". CYP2D6 poor metabolizers cannot metabolize atomoxetine, and therefore, the dosage should either be reduced as per guidelines in the product label, or the drug should be contraindicated in these patients. Over dosage can result in undesired hyperactivity, appetite loss and suicidal ideations (Kircheiner et al 2004).

The following are recommended guidelines for atomoxetine prescriptions:

- Extreme caution on use of drug for double null carriers
- Precautionary assessment of drug interactions for carriers, CYP2D6 inhibitors (paroxetine, fluoxetine) may phenocopy PM
- Reduce initial dosage for children and adolescents utilizing double carriers status as indicator of PM phenotype, using the algorithm specified in the drug label

	<70 kg body weight	>70 kg body weight
EM	1.2 mg/kg/day	80 mg/day
PM	0.5 mg/kg/day	40 mg/day

It should be noted, however, that there are no independent studies of atomoxetine and CYP2D6. An article from the manufacturer, Eli Lilly, *(Clin Pharmacokinet 2005; 44: 571-90)* describes how CYP2D6 affects drug levels but does not influence ADRs. Such controversial data is thoroughly evaluated by the FDA, and the information on the drug label should be the ultimate arbitrator of prescriptive guidance from metabolizer status.

IRINOTECAN (Camptosar) as a Model

Recommendation 6: Genotyping for UGT1A1 should be performed as part of the regimen for prescribing irinotecan (Camptosar). It is also a model for other drug-related dosing applications

Rationale. On Aug. 22, the FDA approved a molecular assay (Invader UGT1A1, made by Third Wave Technologies, Inc.) for use in identifying patients that may be at increased risk of adverse reactions to the chemotherapy drug irinotecan HCl (Camptosar) used in the treatment of colorectal cancer. The test detects and identifies specific mutations in the gene that produces UDP-glucuronosyltransferase 1A1 (UGT1A1), an enzyme that conjugates the active metabolite of irinotecan (SN-38) to form a glucuronide metabolite. Clinical studies have shown the assay to be 100% accurate compared with DNA sequencing, the standard for genotype determination (n = 285, 95% lower limit on confidence = 99%). UGT1A1 activity is reduced in individuals with polymorphisms of the UGT1A1*28 allele, which is homozygous in approximately 10% of the North American population. In a prospective study of 66 irinotecan-treated patients, the mutation was associated with a five-fold increase in the risk of drug-related toxicity related to increased blood levels.

According to recent updates in the safety labeling for irinotecan, a one-level reduction in initial irinotecan dose should be considered in patients known to be homozygous for the UGT1A1*28 allele. Because the precise dose reduction in this patient population is not known, subsequent dose modifications should be considered based on individual patient tolerance to treatment. The FDA noted that the assay is intended for use as an aid in making individualized patient treatment decisions and is not a substitute for a physician's judgment and clinical experience. Other important factors such as liver and kidney function, age, and co-administered drugs should also be considered.

e) What role should the clinical laboratory play in developing the use of PGx testing

Recommendation 7: A CLIA-certified clinical laboratory should serve as a reference source for clinical correlations of the DNA variability.

Rationale. Clinical correlations are drawn from in vitro and in vivo research as well as from clinical outcomes. The clinical lab should compile these references and make them available to users of the DNA tests.

Examples of in vitro research are:

- Both *2 and *3 alleles of 2C9 show reduced catalytic activity (increased Km) and /or decreased maximum rate of metabolism (decreased Vmax) [Takanashi et al., 2000, Yamazaki et al., 1998].

- The *10 allele of CYP2D6 is a mutation resulting in an unstable enzyme with decreased catalytic activity for debrisoquine [Roh et al., 1996].

Examples of in vivo research are:

- Deletion of the entire CYP2D6 gene is a cause of impaired drug metabolism in poor metabolizers of the debrisoquine/sparteine polymorphism [Gaedigk et al., 1991
- Individuals with extra copies in the CYP2D6 locus were ultra-rapid metabolizers of debrisoquine with metabolic ratio 0.01-0.02 (parent drug/metabolite) [Johansson et al., 1993].
- Individuals who are homozygotes for the *3 allele of CYP2D6 (deletion of one nucleotide, causing a reading frame disruption) have absent 2D6 protein and function and poor debrisoquine metabolism [Kagimoto et al., 1990].
- Individuals who are homozygotes for the *4 allele of CYP2D6 demonstrated the highest mean plasma concentrations of nortriptyline and the lowest mean plasma concentrations of 10-hydroxynortriptyline, compared to subjects with a different number of functional CYP2D6 genes [Dalen et al., 1998].
- The *9 allele of CYP2D6 has been correlated to intermediate debrisoquine phenotype in a family study [Broly et al., 1993].
- Individuals who are carriers of the *17 allele of CYP2D6 were found to be strongly associated with greatly decreased (but not deficient) capacity for debrisoquine hydroxylation [Masimirembwa et al., 1996].
- Individuals who are *17 carriers of CYP2D6 tend to have lower metabolic activity, but the decrease is not homogeneous among substrates. *17 carrier status is associated with at least normal metabolic capacity for risperidone metabolism, which suggests that *17 may influence various drugs differently [Cai et al 2006]
- Individuals who are *3 heterozygotes and homozygotes of CYP2C9 were demonstrated to have 66% and 90% of (S)-warfarin clearance, respectively [Takahashi et al., 1998]
- Individuals who are *3 homozygotes of CYP2C9 are poor metabolizers for warfarin [Steward et al., 1997], acenocoumaral [Verstuyft et al., 2001], tolbutamide [Sullivan-Klose et al., 1996], losartan [Yasar et al., 2002], phenytoin [Kidd et al., 1999], glipizide [Kidd et al., 1999]
- Individuals who are *2 or *3 homozygotes or heterozygotes of CYP2C19 showed significant impairment of phenytoin hydroxylation [Aynacioglu et al., 1999].
- Individuals who are carriers of *2 allele of CYP2C19 individuals demonstrated significantly higher ($p<0.0005$) median omeprazole/5'-hydroxyomeprazole metabolic ratio (3.75 versus 0.94 for the wt) [Kim et al., 2002]
- Poor metabolizers of CYP2C19 (2C19*2/2C19*2, 2C19*3/2C19*3, 2C19*2/2C19*3) demonstrated 92-fold greater mean area under the plasma concentration-time curve (AUC) of R-mephobarbital (R-MPB) than wt (2C19*1/2C19*1) [Kobayashi et al., 2004].

Examples of clinical outcomes research are:

- Individuals who are CYP2D6 poor metabolizers have a higher rate of some adverse effects of atomoxetine [PDR 2005]
- Individuals who are *2 or *3 carriers of CYP2C9 require lower maintenance dose of Warfarin, and experience a first bleeding event sooner with standard unadjusted dosages, consistent with a poor metabolizer phenotype [Higashi et al., 2002]
- Individuals who are *2/*2 or *2/*3 poor metabolizers of CYP2C19 showed higher cure rate (100% vs 60% in wt) of gastric ulcers in patients treated with omeprazole and amoxicillin, suggesting that genotyping test for CYP2C19 seems to predict cure of H. pylori infection and peptic ulcer [Furuta et al., 1998].

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VII. TDM AND PHARMACOGENETICS INTERFACE CONSIDERATIONS

(Authors: L. Shaw and G. Burckart; Reviewer: M. Linder)

General introduction. The major role of laboratory medicine and clinical laboratories in support of pharmacotherapy has been in the area of therapeutic drug monitoring (TDM) (ref TDM guidelines NACB). Medications which require monitoring of their concentrations in blood are those where narrow therapeutic concentration ranges in blood are required for efficacy, (e.g., digitalis, some aminoglycosides, tacrolimus, methotrexate, lithium)[A-E], and where toxicity is a persistent problem. As the discipline of pharmacogenetics (PGx) finds its way into clinical practice, the combination of traditional TDM and PGx must be explored to achieve optimum utilization of the combined information they provide. In essence, PGx provides information that allows the clinician to make a determination of appropriateness and risk of drug therapy prior to the initiation of therapy. PGx may then have an additional place in selecting drug or dosage alterations during the treatment for a disease process.

The evaluation process in TDM is essentially a phenotyping procedure that globally reflects the state of the patient's disease state, or represents the pharmacokinetics (PK) and pharmacodynamics (PD) of a drug or drug regimen. Since TDM is not just drug levels alone, the broader field of pharmacogenomics (PGx) may also play a role in TDM in the future. The combination of both PGx and TDM can be powerful because they are more complementary than mutually exclusive, as supported below.

Questions for consideration

a) Will use of PGx information preclude or require TDM in future? and, How should standard TDM practices be modified to account for pharmacogenetic variation?

b) Are there specific clinical situations demonstrative of both TDM and PGx information having complementary value?

c) How can TDM be best utilized in establishing the predictive value of PGx tests, as end point, etc.?

Recommendations

a) Will use of PGx information preclude or require TDM in future? and, How should standard TDM practices be modified to account for pharmacogenetic variation?

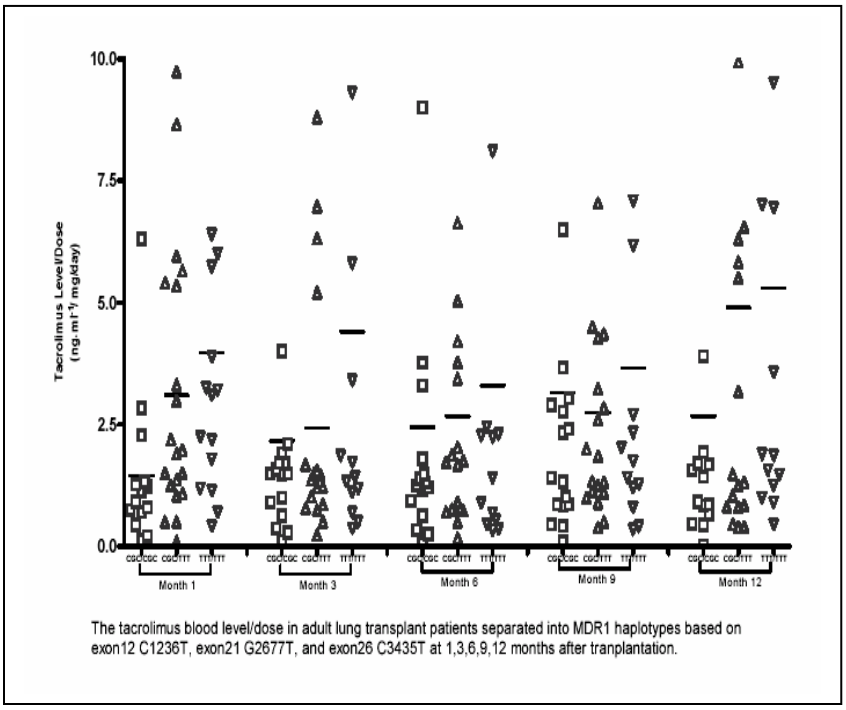
b) Are there specific clinical situations demonstrative of both TDM and PGx information having complementary value?

Recommendation 1. It is recommended that PGx-tests information be used for the initial selection of drugs or doses for some agents with the caveat that the clinician must realize that significant variability may still be observed when using PGx information to design a drug regimen and that TDM is still essential to monitor the response and

toxicity of the PGx-designed regimen. Thus, drug concentrations in serum should still be measured whenever possible.

Rationale 1. Statistically significant associations with genetic polymorphisms inherently have extreme variability in relation to many patient outcome parameters. The best documented of those parameters are between genetic polymorphisms and drug pharmacokinetics. Two examples of this include:

(a) Tacrolimus in organ transplantation, as discussed in "Burckart GJ, Liu XI. Pharmacogenetics in transplant patients: Can it predict pharmacokinetics and pharmacodynamics? *Therapeutic Drug Monitoring* 2006;28:23-30"..



The tacrolimus blood level/dose in adult lung transplant patients separated into MDR1 haplotypes based on exon12 C1236T, exon21 G2677T, and exon26 C3435T at 1,3,6,9,12 months after tranplantation.

Figure 1. The effect of *ABCB1* haplotypes (CGC/CGC, CGC/TTT, and TTT/TTT) on tacrolimus level/dose in lung transplant patients over the first year post transplantation. The horizontal lines represent the mean tacrolimus level/dose and a significant relationship does exist between *ABCB1* haplotype and tacrolimus level/dose over the first postoperative year. However, the raw data is presented to demonstrate that the mean data cannot be used to predict the tacrolimus level/dose on any individual patient.

(b) Warfarin dosing as discussed in "Scordo MG, Pengo V, Spina E, Dahl ML, Gusella M, Padrini R. Influence of CYP2C9 and CYP2C19 genetic polymorphisms on warfarin maintenance dose and metabolic clearance. Clinical Pharmacology and Therapeutics 2002;72:702-710.

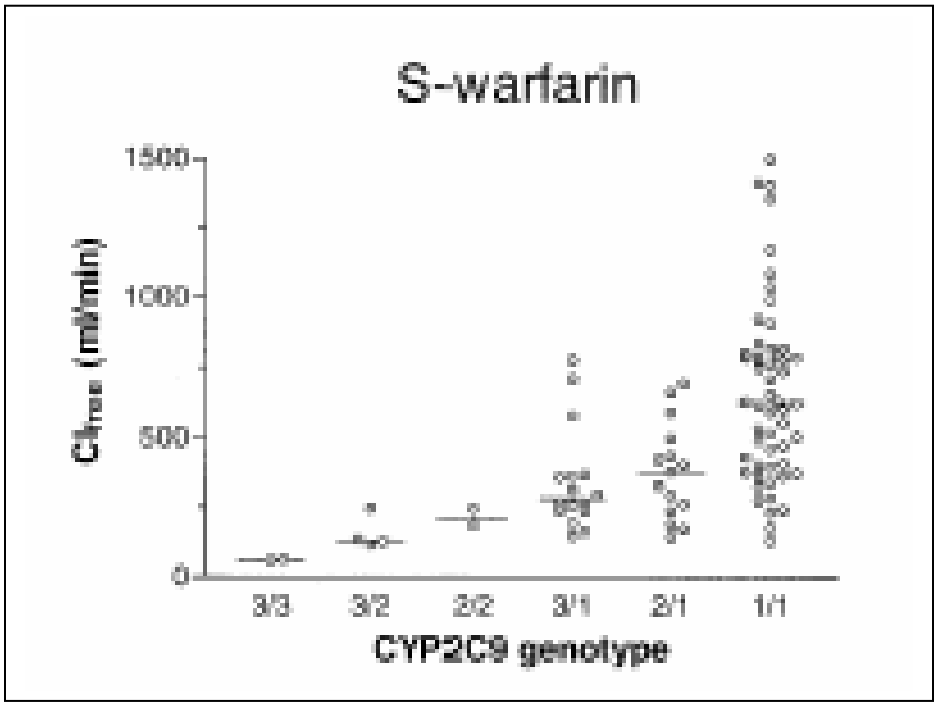


Figure 2. Correlations between *S*-warfarin unbound oral clearance (CLfree) and *CYP2C9* genotype .

Rationale 2: Plasma drug concentrations are a valuable asset for evaluating pharmacogenetic variables. In the case of pharmacogenetic variants which influence drug pharmacokinetics these measurements can facilitate the estimation of fundamental pharmacokinetic parameters. These pharmacokinetic parameters can facilitate genotype guided dosing strategies to achieve normalized drug exposure between genotypes and allow for design of more appropriate monitoring strategies. This is arguably a valuable clinical end point as there are clearly associations between excessive plasma drug concentration and increased risk of adverse drug reactions (Atomoxetine as an example). Modification of dosing practices to achieve plasma drug concentrations that have been established as safe and effective for EM's is an excellent approach to deriving the much needed genotype guided dosing strategies and for estimating the improvement of pharmacogenetics in drug safety.

Pharmacodynamic variables may also be better defined by studies that incorporate plasma drug concentration measurements. For example, what is the basis for the association between the -1639 promoter polymorphism of VKOR and decreased warfarin dose requirements? It has been hypothesized that this variant decreases VKORC1 transcription and therefore limits the production of this protein. However there are conflicting reports on this issue. Zhu (in preparation) has recently demonstrated statistically significant differences in steady state S-warfarin plasma concentrations in stabilized patients between individual with the VKORC1 -1639 GG , GA and AA genotypes. (Zhu, 2006, in preparation)

c) How can TDM best be utilized in establishing the predictive value of PGx tests, as end point, etc.?

Recommendation 2: When the pharmacogenetic characteristic results in a change in drug pharmacokinetics or receptor sensitivity, TDM practices should take into account necessary changes in time to reach steady-state plasma concentrations and drug clearance, as well as the need to adjust the typical therapeutic range for a drug according to the genotype.

Rationale. In order to properly evaluate the plasma drug concentration that is established by a dosing schedule, the patient must have achieved a steady-state plasma drug concentration. Most standardized therapeutic drug monitoring protocols are designed to collect specimens once the patients are assumed to have reached steady-state which is the time equal to ~ 5 elimination half-lives of the medication. Individuals with a genetic variant which decreases or increases the elimination half-life of the medications will also have an increased or decreased time to steady-state. Therefore standard TDM practices must be modified to account for this change in time.

Similarly, when the medication receptor abundance or affinity for the medication is altered as a consequence of genetic variation, the plasma drug concentration required to elicit the desired therapeutic response may have to be adjusted to concentrations outside of the typical therapeutic range.

For example, standard practice of initial dose adjustments for the anticonvulsant phenytoin call for an initial measurement of phenytoin plasma concentration on the third day of therapy. This practice is based on the fact that the average half-life of phenytion is < 14 hrs and therefore the patient is expected to be nearing a steady-state plasma concentration for that dose within ~ 72 hours. In contrast an individual with a genetic deficiency in cytochrome P4502C9 will have a phenytoin elimination half-life of > 30 hours and therefore the plasma concentration measured on the third day of therapy will be much lower than the steady state concentration achieved after ~ 6 days of dosing.[A]

As another example, atomoxetine elimination is ~ 4 hours in subjects who are CYP2D6 extensive metabolizers.[B] As is true for drug accumulation to steady-state, drug wash out is also directly linked to the elimination half-life of the drug. Therefore, steady-state plasma concentrations will be achieved within the first day of therapy and upon discontinuation of atomoxetine, the atomoxetine is expected to be > 95% eliminated within 24hrs of discontinuation and an alternative therapy can safety be initiated. To contrast this with a CYP2D6 PM where the atomoxetine also given standard atomoxetine dosages. Steady-state plasma concentrations are not achieved until approximately 4 days of therapy and when achieved are ~ 5-times higher than anticipated for EM's. Further upon discontinuation, plasma concentration is still well above expected peak concentration for EM's at 24 hrs and has not returned to baseline until more than 3 days following discontinuation.

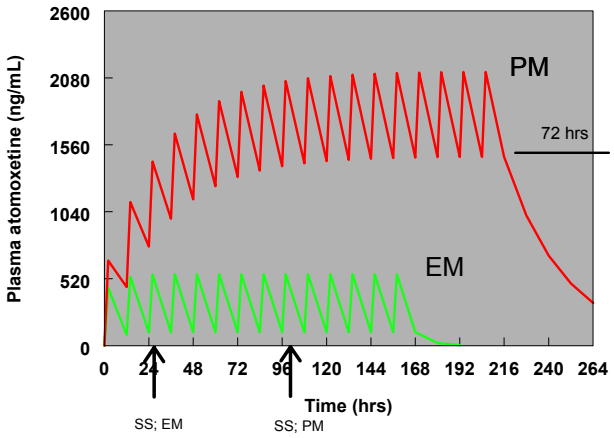


Figure 3. Atomoxetine plasma accumulation models for CYP2D6 EM versus PM subjects. Models are based on 20 mg bid dosing and published pharmacokinetic parameters for both genotypes.

An example of the potential necessity to change the therapeutic range for a given genotype is the recently reported genetic variation in the vitamin K epoxide reductase complex 1 protein.[C] This protein has shown that the variant has no effect on warfarin clearance, but lower dosages are adequate to establish the target level of anti-coagulation. This is presumably due to the fact that lower plasma concentration of the S-warfarin enantiomer are adequate to inhibit this complex because of a lower abundance of the protein.

Recommendation 3: Plasma drug concentrations should be measured in all pharmacogenetic studies.

Rationale: Plasma drug concentrations are a valuable asset for evaluating pharmacogenetic variables. In the case of pharmacogenetic variants which influence drug pharmacokinetics these measurements can facilitate the estimation of fundamental pharmacokinetic parameters. These pharmacokinetic parameters can facilitate genotype guided dosing strategies to achieve normalized drug exposure between genotypes and allow for design of more appropriate monitoring strategies. This is arguably a valuable clinical end point as there are clearly associations between excessive plasma drug concentration and increased risk of adverse drug reactions (Atomoxetine as an example). Modification of dosing practices to achieve plasma drug concentrations that have been established as safe and effective for EM's is an excellent

approach to deriving the much needed genotype guided dosing strategies and for estimating the improvement of pharmacogenetics in drug safety.

Pharmacodynamic variables may also be better defined by studies that incorporate plasma drug concentration measurements. For example, what is the basis for the association between the -1639 promoter polymorphism of VKOR and decreased warfarin dose requirements? It has been hypothesized that this variant decreases VKORC1 transcription and therefore limits the production of this protein. However there are conflicting reports on this issue. Had Sconce et al and others who have reported on this genetic variant measured the steady-state S- and R- warfarin concentrations it is likely that they could have confirmed the suspicion that the lower dose requirements are consistent with lower effective plasma concentrations in subjects with the -1639 AA VKORC1 genotype. Likewise, if earlier studies to establish the role of CYP2C9 on warfarin safety had included plasma warfarin concentrations in all studies, the fact that some patients required lower dosages and had lower plasma drug concentration could have accelerated the discovery of a pharmacodynamic basis for low warfarin dose requirements.

Recommendation 4. PGx and TDM play complementary roles in managing patients on drug therapy. This interface occurs not only when initiating drug therapy, but is also critically important when making adjustments to a therapeutic regimen whether it is a change in dosage or drug. The broader field of PGx will be a critical part of TDM in the future as a measure of the response of the disease to the drug therapy or as an indicator of drug toxicity.

Rationale. Given the variability supported in Recommendation 1, then it is logical to assess the outcome after drug or dosage selection is performed. This assessment may be made through TDM procedures including drug levels, but may also be made in the future using PGx assessment tools such as gene profiling in peripheral blood. Additional support for the need of assessing outcome after drug selection and administration comes from the fact that no work has been performed with PGt and drug and food interactions. Therefore, the magnitude of drug interactions cannot be predicted based upon PGx, and therefore TDM including PGx testing is necessary to measure outcomes and to reassess therapy. Support from studies in lung transplantation is discussed in "Burckart GJ, Hutchinson IV, Zeevi A. Pharmacogenomics and lung transplantation: Clinical implications. The Pharmacogenomics Journal (in press)".

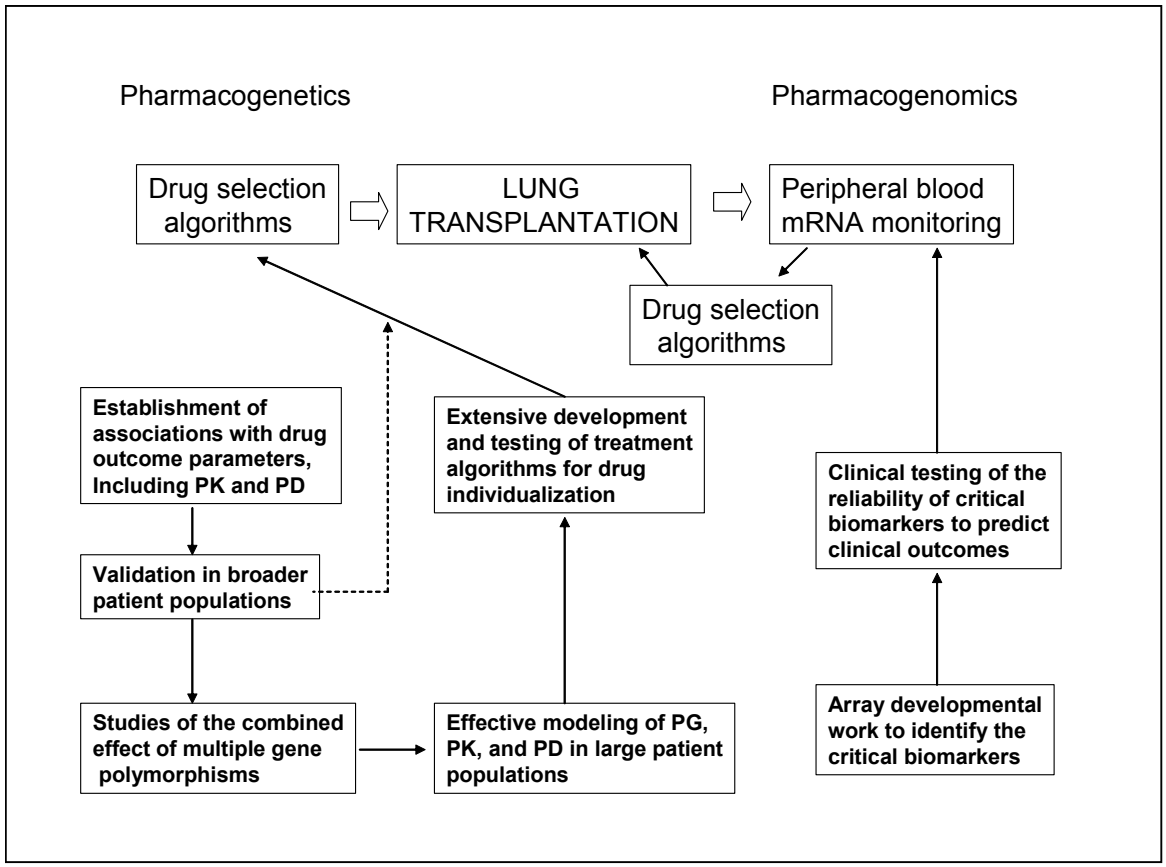


Figure 4. Graphic depiction of the steps involved with the development of pharmacogenetics and pharmacogenomics in lung transplant patients. The left side indicates the route for development of pharmacogenetic associations, and the route may be shortcut at the point of the dashed line for polymorphisms that are heavily weighted for impact on patient outcomes. The right side demonstrates the development of pharmacogenomic monitoring in lung transplant patients. Both sides come together in the treatment and monitoring of the lung transplant patient.

(a) Support for the complexity of drug interactions and the lack of pharmacogenetic information in predicting those drug interactions comes from "Warrington JS and Shaw LM. Pharmacogenetic differences and drug-drug interactions in immunosuppressive therapy, Expert Opin. Drug Metab. Toxicol 2005;1:1-17". See Table below. Clinical drug-drug interactions between immunosuppressants and concomitant medications.

Table 1. Clinical drug-drug interactions between immunosuppressants and concomitant medications

Drugs	Drug classes and examples	Effect
CSA	*Antibiotics*	
	Chloramphenicol	↑concentrations
	Clarithromycin	↑concentrations
	Doxycycline	↑concentrations
	Erythromycin	↑concentrations
	Trimethoprim	↓concentrations
	Antidepressants	
	Nefazodone	↑concentrations, toxicity
	Fluvoxamine	↑concentrations, toxicity
	Antiepileptics	
	Phenobarbital	↓concentrations
	Phenytoin	↓concentrations
	Primidone	↓concentrations
	Antifungals	
	Itraconazole	↑concentrations
	Ketoconazole	↑concentrations, 2-fold
	Calcium-channel blockers	
	Diltiazem	↑concentrations
	Nicardipine	↑concentrations
	Verapamil	↑concentrations
	H2-antagonists	
	Cimetidine	↑concentrations
MPA	*Polyvalent absorbent resins*	
	Sevelamer	↓concentrations, - 20%
	Immunosuppressants	
	CsA	↓concentrations
TAC	*Antibiotics*	
	Erythromycin	↑concentrations, toxicity
	Antiepileptics	
	Phenobarbital	↑elimination
	Antifungals	
	Itraconazole	↑concentrations, toxicity
	Ketoconazole	↑concentrations, toxicity
	Calcium-channel blockers	
	Diltiazem	↑concentrations, toxicity
	HIV protease inhibitors	

	Ritonavir/lopinavir	**↑concentrations, 8-fold**
	Ritonavir/saquinavir	**↑concentrations, 5-fold**
SIR	***Antibiotics***	
	Erythromycin	**↑concentrations, 5-fold**
	Antifungals	
	Ketoconazole	**↑concentrations**
	Antituberculosis agents	
	Rifampin	**↓concentrations**
	Calcium-channel blockers	
	Diltiazem	**↑concentrations**
	Verapamil	**↑concentrations**
EVE	***Antibiotics***	
	Erythromycin	**↓clearance, - 20%**
	Azithromycin	**↓clearance, - 20%**
	Antifungals	
	Itraconazole	**↓clearance, - 75%**
	Calcium-channel blockers	
	Nifedipine	**↔**
	Diltiazem	**↔**
	Verapamil	**↔**
AZA	***Gout medications***	
	Allopurinol	**↑concentrations**
	Salicylates	
	Sulfasalazine	**↑concentrations**
	Salicylic acid	**↑concentrations**

AZA: Azathioprine; CSA: Cyclosporin; EVE: Everolimus; MPA: Mycophenolic acid; SIR: Sirolimus; TAC: Tacrolimus

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VIII. ANCILLARY APPLICATIONS (drug prescription /dispensing, forensics)

(Authors and Reviewers, see two sections below)

General Introduction. Pharmacogenomics has recently been focused on enabling the targeted practice of drug selection by healthcare providers. However, other applications of PGx are evolving beyond that domain, categories include applications in: production of targeted drugs by pharmaceutical companies; forensics; safer distribution of medications by pharmacist; environmental toxicology (toxicogenomics); predicting addiction to substances; and multiple other applications. The information is still much too fresh and evolving rapidly in many of these areas so they by in-large cannot be considered at this time for suggesting specific laboratory medicine application guidelines. However, in the area of both forensics and drug dispensing some recommendations are worthy of consideration because of their eminent applications.

A. Applications in Dispensing of Medications

(Authors: S. Jortani and H. McLeod)

Questions for consideration are:

a) Should information related to PGx test availability be made part of the information provided to patients as part of the drug dispencing mechanism? If so, by whom or how.

b) Should PGx test information be considered an integral part of the drug-dispensing safety awareness practice.

c) Should information-related relationships be fostered between drug dispensing providers and clinical laboratories providing PGx-testing services?

RECOMMENDATIONS

a) Should information related to PGx test availability be made part of the information provided to patients as part of the drug dispencing mechanism? If so, by whom or how.

b) Should PGx test information me considered an integral part of the drug-dispensing safety awareness practice.

Recommendation 1: After appropriate consent from the patient, PGx genotype information should be made available to drug-dispensing organizations to be used as part of their drug-dispensing safety verification procedures.

Rationale: Two areas of importance relative to application of PGx information provided by clinical laboratories and likely also useful to pharmacists are the availability of genotype-information for purposes of alerting either physicians or patients of possible drug interactions and linking that information to dispensing either at remote locations or in hospital settings.

Relating information on drug-drug interaction has become routine in adding to the safety of dispensing medications. The addition of genotyping information able to identify slow or rapid metabolizers (and eventually receptor-related genotyping) may assist in cross-checking not only the efficacy of the drug-type ordered, but also appropriate dosage and possibility of interaction with other concurrent medications that may be concentration-dependent.

c) Should information-related relationships be fostered between drug dispensing providers and clinical laboratories providing PGx-testing services?

Recommendation 2: Hospital-based drug dispensing departments and clinical laboratories should work in close collaboration and establish policies to make available timely genotyping information useful for guiding the dispensing of medication for hospitalized patients and for recommendations after discharge.

Rationale. Presently, clinical laboratory results by way of therapeutic drug monitoring and by measuring other biochemical physiological parameters (creatinie - renal function, etc.) provide pharmacokinetic information to pharmacy departments and thus assist in optimizing drug administration. This typically requires close communication between the clincal laboratories and the dispening units. In a similar manner, a close association between the clinical laboratory and the drug-dispensing entity at hospitals and clinics should be fostered and policies should be established to encourage and permit this cooperation.

B. Applications in Forensics

(Authors: S. Jortani and S Wong; Reviewer: A Wu)

The clinical use of genetic testing for purposes of dosing drugs and evaluation of potential toxicity to various therapeutics such as the anticoagulants, antidepressants, antipsychotics, and pain management such as opioid drugs is rapidly gaining popularity. (Eap 2001, Sadee 1999, Shi 2006, Wong 2000.) These groups of drugs are often identified as leading causes of death.(Anderson 2000, Cooke 2001, Drummer 1994, Gagajewski 2003, Kuhlman 2003, Lilleng 2004, Mikolaenko 2003, Milroy 2000, Winecker 2003, Wong 2000.) In light of intense emphasis on this particular discipline, practitioners in forensic toxicology investigations have also considered its use in interpreting drug poisoning cases (Bailey 2000, Druid 1999, Holmgren 2006, Jannetto 2002, Jin 2005, Levo 2003, Sajantila 2006, Sallee 2000, Shi 2006, White 2005, Wong 2002, 2003, 2005, 2006.) Considering the fact that information gained through pharmacogenetic testing on a deceased individual may also impact the surviving family, issues regarding confidentiality and health information should be considered by the laboratories. In order to make sure several sampling, testing and interpretive considerations in forensic implementation of such testing, we propose the following questions and recommendations:

Questions for consideration are:

a) In forensic applications of PGx-testing, what is (are) the preferred specimen(s), and what diligence should be established for purposes of evidence acquisition ?

b) What type of information and correlations should be used to optimize the application of PGx data in forensic cases?

c) What qualifications by way of training and experience should be required for individuals reporting and interpreting PGx information when applied to forensics?

d) What type of information should accompany a PGx test report as it applies to applications in forensics?

e) Are there any particular or specific ethical considerations that may apply to the use of PGx data with regard to applications in forensics?

Recommendations

a) In forensic applications of PGx-testing, what is (are) the preferred specimen(s), and what diligence should be established for purposes of evidence acquisition?

Recommendation 1: For forensic purposes, blood is considered to be the preferred specimen of choice and should be used whenever available.

Rationale: Many commercial pharmacogenetic tests have been validated for whole blood (Druid 1999, Prouty 1990) . Other samples such as tissues, buccal swabs and saliva are also being used to a lesser extent. Alternative tissue samples may be used if they have been shown to yield the required DNA amount needed for testing (Hochmeister 1991). Extraction, amplification and validity of testing alternative specimen should have been previously established before their use in testing forensic samples (Druid 1999, Levo 2003).

Recommendation 2: Chain of custody should be maintained for forensic samples according to the established protocols by each laboratory.

Rationale: As required by legal proceedings, such samples should be stored at 4 degrees C in a locked cabinet until testing. The extracted and amplified genomic DNA should also be stored at -70 degrees C for a minimum of 2 years after testing (Druid 1999, Jannetto 2002, Jin 2005, Wong 2002). .

b) What type of information and correlations should be used to optimize the application of PGx data in forensic cases?

Recommendation 3: Whenever possible, in cases in which polymorphic enzymes are suspected as factors in drug toxicity, other relevant issues such as polymorphisms in receptors, transport proteins, genes that affect pharmacodynamics, etc. should also be considered.

Rationale: If drug poisoning is suspected as indicated by case history and/or autopsy, measurement of blood and tissue concentrations of drug and its metabolites is routinely performed (Druid 1999, Ggajewski 2003, Jannetto 2002, Jin 2005, Levo 2003, Shi 2006, Wong 2002, 2003). After case review of drug toxicity involving polymorphic enzymes, genotyping for these particular enzymes and proteins is recommended. The latter will assist, as an adjunct, in interpretation of cause of overdosage and should not be used on its own to establish cause of death (Druid 1999, Holmgren 2006, Jannetto 2002, Jin 2005, Kuhlman 2003, Levo 2003, Sajantila 2006, Sallee 2000, Wong 2002, 2003, 2005, 2006) .

c) What qualifications by way of training and experience should be required for individuals reporting and interpreting PGx information when applied to forensics?

Recommendation 4: Interpretation of pharmacogenetic testing results in forensic toxicology should be done by toxicologists with adequate training in pharmacogenetic testing and familiarity with metabolic pathways

(Druid 1999, Holmgren 2006, Jannetto 2002, Jin 2005, Levo 2003, Sallee 2000, Shi 2006, White 2005, Wong 2000, 2002, 2003, 2006).

d) What type of information should accompany a PGx test report as it applies to applications in forensics?

Recommendation 5: Reporting of poor heterozygote and ultrarapid phenotypes should be accompanied by information about the degree of a particular polymorphism's role in the pharmacokinetics or dynamics of the drug(s) in question. For example, reporting of a poor-metabolizer phenotype should include documentation of the impact of such phenotype on handling of a drug.

(Druid 1999, Jannetto 2002, Jin 2005, , Sagjantila 2006, Sallee 2000, White 2005, Wong 2000, 2002, 2003, 2006).

e) Are there any particular or specific ethical considerations that may apply to the use of PGx data with regard to applications in forensics?

Recommendation 6: Ethical consideration — Currently, IRB and informed consent of the decedent family are not required for postmortem analysis. However, consideration and consultations with the supervising legal authorities/medical management would be advised in order to maintain the high standard of ethics to preserve the rights of the decedent and family members.

(Jannetto 2002, Jin 2005, Levo 2003, Sajantila 2006, White 2005, Wong 2000, 2002, 2003).

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IX. REGULATORY CONSIDERATIONS

(Authors: F. Frueh / A Rahman; Reviewers: Jerry Collins and Allen Rudman)

General Introduction. Regulatory considerations for the evaluation and approval of pharmacogenomic tests can be categorized into two broad aspects: analytical validation and clinical usefulness of the test. This evaluation is hinged on the mission the FDA fulfills: to advance public health by helping to speed innovations that make medicines more effective and safe. Although tests are evaluated by the Center for Device and Radiological Health (CDRH) and drugs/biologics are evaluated by the Center for Drug Evaluation and Research (CDER), the two centers interact closely and the evaluation of pharmacogenomics test is often carried out in a joint effort, particularly in cases where drugs and tests are co-developed and cross-reference is being made in drug- and device-labels.

Considering the scientific evidence submitted for regulatory approval, the U.S. Food and Drug Administration (FDA) creates, in cooperation with the applicant, a label (package insert) that describes the analytical and clinical characteristics of the product (i.e. a drug or a device). For example, a label for a drug that should only be taken by a specific subpopulation, should describe the scientific evidence why this drug is indicated for this population (i.e. efficacy) and/or why it is not indicated for other populations (i.e. safety and/or efficacy). In addition, the label should also provide information about the marker for which a test is indicated and, if available, the test itself. Together, this information will allow the healthcare professionals and the consumer to make better decisions about treatment, helping to answer questions such as "will this drug work?" or "what is the best dose of this drug ?". Hence, the drug labels reflect a "manual" for the drug or device, in which information is provided about how to best use the product based on our current understanding of the science associated with the product.

Even though a significant number of (i.e. more than 100) labels for drugs and devices contain pharmacogenetic or pharmacogenomic information, it needs to be realized that this knowledge is often new and evolving. Consequently, and as it is the case for other, non-genomic information, it is not always possible to strictly require certain actions based on current genetic or genomic information. For example, even though there is scientific evidence indicating that erlotinib is less beneficial in EGFR-negative patients, the current label for Tarceva® does not require this test in order for the drug to be prescribed and the drug is available to everybody. In contrast, the Her2/neu antibody trastuzumab has shown to be efficacious in Her2/neu-positive breast cancers and was consequently studied only in this population, which allowed for a more restricted label pointing out that a positive Her2/neu test is required before Herceptin® can be prescribed. To accommodate this variation in scientific evidence, the FDA considers the use of genomic information in drug labels to either require, or recommend the use of a genomic test prior to drug therapy, or simply provides information about the current knowledge of genomics that is relevant to drug therapy without the requirement or recommendation of a specific action. Examples of this, the level of evidence, and the tests relevant to the examples are considered in the following questions and recommendations as applied to information provided by clinical laboratories.

Questions for consideration are:

a) When will a test be "required", when is a test "recommended"?
b) When should the label state that the test is "available"?

Recommendations

a) When will a test be "required", when is a test "recommended"?

Recommendation 1: A test may be required for a therapy when the drug or the biologic is co-developed with a test. Patients are eligible to receive a treatment only if a test result is obtained prior to treatment initiation.

Rationale: Certain targeted therapies may only benefit a population that expresses maker for response. A testing for HER2/Neu protein (human epidermal growth factor receptor 2 protein) over-expression is required prior to the use of trastuzumab alone or in combination with other agents for metastatic breast cancer. Trastuzumab is a recombinant DNA-derived monoclonal antibody that selectively binds to the extra domain of HER2. Clinical trials for approval of trastuzumab were conducted in patients whose tumor over expressed the HER2 protein, and clinical benefit of trastuzumab over other chemotherapy was established only in HER2 over expressed population. Similarly, cetuximab is indicated for the treatment of patients with human epidermal growth factor receptor (EGFR) expressing metastatic colorectal cancer. Patients enrolled in the clinical trials were required to have immunohistochemical evidence of positive EGFR expression in their primary tumor or tumor from a metastatic site. Specimens were scored based on the percentage of cells expressing EGFR and the intensity of expression. Lesko LJ Nat Rev Drug Discov. 2004, FDA Website: www.fda.gov/cder/genomics

Recommendation 2: The test may be recommended prior to the selection of a therapy and/or the selection of a dose for a particular population deficient in activity of a polymorphic enzyme involved in the inactivation of the drug/biologics.

Rationale: Toxicity of a drug/biologics may be associated with the inability of the body to inactive the agent through metabolism. Irinotecan is indicated for the treatment of metastatic colorectal cancer. Initial irinotecan dose reduction is recommended for patients who are deficient in UGT1A1 (Uridine diphosphate-glucuronosyl transferase 1A1) enzyme required for inactivation of the active metabolite of irinotecan, SN-38. Patients deficient in UGT1A1 activity are at increased risk for severe neutropenia resulting in hospitalization, dose reduction and/or treatment delay. Testing for TMPT (Thiopurine methyltransferase) status prior to or during 6-mercaptopurine or azathioprine treatment is recommended to avoid bone marrow related severe toxicity. Patients with two non functional alleles have low or absent TPMT activity resulting in accumulation of toxic metabolites causing life-threatening myelotoxicity if they receive normal dose of 6-mercaptopurine or azathioprine. Lesko LJ Nat Rev Drug Discov. 2004, FDA Website: www.fda.gov/cder/genomics

b. When should the label state that the test is "available"?

Recommendation 1: The availability of a test may be mentioned in the label of a drug/biologics to inform the patients and the health care provider about the availability of the test.

Rationale: Information about the availability of a test to detect the genetic defect of an enzyme associated with the deactivation of the drug/biologics may be included in the label when a drug/biologics has a broad therapeutic window and a normal dose does not compromise with the safety and effectiveness of the agent. Atomoxetine is predominantly metabolized by CYP2D6. The drug is titrated to a maximum dose of 1.2mg/kg and has a wide therapeutic window. Low starting dose allows for a safe dose titration to a desirable therapeutic effect in patients with CYP2D6 deficiency. The Laboratory Test section of the atomoxetine drug label includes information about the availability of laboratory tests to detect the poor metabolizers of CYP2D6. Since the use of the test is unlikely to have any clinical impact on dosing of this drug for CYP2D6 poor metabolizers, inclusion of the test in the package insert is for information purpose only.

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X. GLOSSARY - Definitions useful in understanding pharmacogenetics

(Author: D. Farkas)

allele version of a gene at a given locus

amplicon copy of a target DNA created by PCR or other *amplification* methods

central dogma (of molecular biology) fundamental tenet of molecular biology stating that DNA is copied via *replication*, RNA is derived from DNA via *transcription*, and protein is derived from RNA via *translation.* The discovery of reverse transcription disrupted the central dogma of molecular biology by showing that genetic information could also flow from RNA to DNA, not just DNA to RNA.

complementary DNA (cDNA*)* DNA produced using an RNA template via the enzyme *reverse transcriptase*

compound heterozygote individual with two abnormal *alleles* at a given locus, each with a difference *polymorphism* or *mutation*.

deletion *mutation* resulting from the removal of base(s)

DNase enzyme that degrades DNA

DNA ligase enzyme that joins two pieces of DNA

DNA polymerase enzyme that uses DNA as a template to produce a complementary strand of DNA; *cDNA* is made with a type of DNA polymerase called *reverse transcriptase* (an RNA-dependent, DNA polymerase)

DNA sequencing base-by-base determination of the exact sequence of target DNA

epigenetic referring to heritable changes to the *genome* that do not alter the coding sequence

frame-shift mutation insertion or deletion of base(s) that alters the reading frame of a coding sequence, thereby changing the amino acids encoded downstream and/or producing a stop codon

gel electrophoresis separation of DNA by size via migration in an electric field in an agarose or polyacrylamide matrix

gene segment of DNA transcribed into RNA that (i) is translated into a protein or (ii) forms structures such as ribosomes

genetic variant alternative forms of a gene which may or may not lead to altered phenotype

genome all the genetic material of an organism

genotype the *alleles* at a given locus in an individual; see also *phenotype*

haplotype analogous to genotype, haplotype is the set of alleles (or SNPs) on one chromosome or part of a chromosome that are linked and usually or often inherited together

heterogeneous regarding PCR, requiring separate amplification and detection steps

homogeneous regarding PCR, having combined amplification and detection steps

hybridization process of forming a double-stranded molecule from a single-stranded *probe* and a single-stranded nucleic acid target

hybridization probe type of fluorescently-labeled *probe* used in *real-time PCR* that produces signal following *hybridization* to a target DNA

missense mutation base change resulting in coding of a different amino acid

molecular diagnostics diagnosis of disease using nucleic acids as analytes, often used synonymously with *molecular pathology*

mRNA messenger RNA, *translated* into protein

mutation generally harmful DNA sequence change; compare to *polymorphism*

nucleic acids deoxyribonucleic acid (DNA) and ribonucleic acid (RNA), the molecules containing the genetic code

nucleoside *nucleotide* lacking a phosphate group

nucleotide building block of *nucleic acids* composed of phosphate group(s), a five-sided sugar molecule, and a nitrogenous base

oligonucleotide short sequence of *nucleotides*, often used as primers for PCR or *DNA sequencing*

penetrance percent expression in a population of the *phenotype* of a given mutant *genotype*

pharmacogenetics the hereditary basis for inter-individual differences in drug response

pharmacogenomics the convergence of pharmacogenetics and genomics used to mean the influence of DNA sequence variation on the effect of a drug on an individual

phenotype traits resulting from a given *genotype*

point mutation *mutation* that changes a single base

polymerase chain reaction (PCR) enzymatic *in vitro* nucleic acid *amplification* method using temperature cycling to produce repeated cycles of DNA *replication*

polymorphism variant DNA sequence change, typically benign, found in 1% or more of individuals; compare to *mutation*

primer *oligonucleotide* used in *PCR* or *DNA sequencing* to target an area of interest

primer-dimers nonspecific products formed during *PCR* by the interaction of *primers*

probe relatively small piece of DNA or RNA used to find or detect a specific piece of target nucleic acid

proteomics study of the entire complement of proteins in organisms

quantification standard synthetic nucleic acid standard spiked into samples before processing to serve as a reference in quantitative *PCR*

real-time PCR *PCR* in which detection of product is simultaneous with *amplification*

replication process of duplicating DNA with *DNA polymerase*

restriction endonuclease (RE) enzyme purified from bacteria that recognizes and cleaves unique sequences

restriction fragment length polymorphism (RFLP) *polymorphism* that changes the electrophoretic banding pattern of DNA fragments generated by digestion with a *restriction endonuclease*

single nucleotide polymorphism (SNP) *polymorphism* that is a single base change

Southern blot hybridization DNA detection method where digested sample is separated by *electrophoresis*, transferred to a membrane, and probed

transcription process of producing *mRNA* from a DNA template

translation process of converting the information contained in *mRNA* into protein

uracil-N-glycosylase enzyme used to prevent *amplicon* carryover contamination that degrades any DNA containing uracil (uracil-containing DNA is not natural and is produced *in vitro* during some PCR protocols)

variant allele specific alternative forms of a gene, generally causing a known alternative phenotype

wild-type normal *allele*; compare to *mutant or variant*

OTHER APPENDICES (here)

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