

## 5. PHARMACOGENOMICS AND PERSONALIZED MEDICINE

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### 5.1 Introduction

Two interwoven processes, human genome sequencing and the development of new technologies using DNA as an analytical sample and as a reagent have resulted in the genetic revolution in different fields of medicine, such as the field of medical therapy leading to personalized medicine through pharmacogenetics approach.

Pharmacogenetics is a newer branch of pharmacological sciences studying the relationship between genetic predisposition of an individual and his ability to metabolize a drug. It helps understand why some individuals respond to drugs and others do not, why some individuals require higher or lower doses to achieve an optimal therapeutic response, and tries to help the physician identify those patients who will respond favorably to therapy or develop side effects.

Systematic studies involving review of published literature indicate that adverse drug reactions (ADR) are prevalent and associated with costly hospitalizations.

Individual variation in response to drug ranges from failure to respond to drug reactions and drug to drug interactions when several drugs are taken simultaneously. The clinical consequences range from patient's discomfort through serious clinical illness to the occasional fatality. Approximately 7% of patients are affected by adverse drug reactions, increasing the overall hospital costs by 1.9% and drug costs by 15%. Some 0.3% of adverse drug reactions have fatal outcomes.

Among other influences, such as physiological, pathophysiological and lifestyle factors, the intraindividual genetic variability has a major impact on drug activity.

### 5.2 Levels of interindividual variability in drug effects

Genetic variability is known for drug absorption, drug metabolism and drug interactions with receptors. This forms the basis for slow and rapid drug absorption, poor, efficient or ultrarapid drug metabolism, and poor or efficient receptor interaction.

Genetic polymorphism based on drug metabolism ability is associated with four phenotype classes. The phenotype of extensive (normal) drug metabolizer (EM) is characteristic of normal population. Individuals are either homozygous or heterozygous for the wild type allele. Individuals that are heterozygous for the wild type allele may have intermediate metabolizer (IM) phenotype and may require lower than average drug dose for optimal therapeutic response. The poor metabolizer (PM) phenotype is associated with the accumulation of specific drug substrates in the body due to mutation and/or deletion of both alleles responsible for phenotypic expression. Individuals with PM phenotype are either homozygotes or multiple

heterozygotes for mutant alleles. The ultrarapid metabolizer (UM) phenotype is characterized by enhanced drug metabolism due to gene amplification. Individuals having this phenotype are prone to therapeutic failure because drug concentrations at normal doses are by far too low. Five to 20% of patients can belong to one of these risk groups, depending on the population studied. It is important to mention that there are significant ethnic and racial differences in the frequency of variant alleles.

### 5.3 Mechanisms of genetic variations

Genetic variations are the result of multiple mechanisms such as insertion, deletion, variable tandem repeats and microsatellites but the most frequent polymorphisms are point mutations or single nucleotide polymorphisms (SNPs), accounting for over 90%. Some of the polymorphisms are without consequences, but others cause altered protein, truncated protein, unstable protein or protein due to expression level. When we talk about a polymorphism, we mean a mutation in the genetic code that occurs in more than 1% of a population.

### 5.4 The potential of pharmacogenetics as a discipline in laboratory medicine

Pharmacogenomics and pharmacogenetics deal with the use of information derived from analysis of gene variations with objective to guide the drugs use. The pharmacogenomics studies are focused on the contribution of multiple genes (or entire genome) to drug response variability, whereas pharmacogenetics is focused on the association between single gene and drug response variability. Single gene testing can include pharmacodynamic genes, such as serotonin transporter or dopamine receptors, but these gene tests are not ready for clinical use yet, however, pharmacokinetic genes such as CYP2D6 and CYP 2C9 have reached clinical practice. Also, testing a limited number of multiple genes is possible by several commercial laboratories, providing batteries of genotype testing such as pharmacokinetic genes CYP2D6 and CYP2C19.

Pharmacogenetics has two functional components that link pharmacology to genetics. One may predict how drugs are processed by the body (pharmacokinetics) and the other how drugs interact with receptors to cause drug response (pharmacodynamics). Pharmacokinetics is strongly linked to biotransformation of drugs by metabolic processes mostly by the liver and their subsequent elimination by kidney function. The pharmacokinetic level includes gene polymorphisms that modify the concentration of a drug and its metabolites at the sites of their molecular action (such as polymorphisms of drug metabolizing enzymes, drug transporters, etc.). However, pharmacodynamics deals with understanding the drug interaction with receptors and the subsequent response. In this process some biotransformation may be involved. The pharmacodynamic level includes gene polymorphisms associated with the drug effect and mechanism of action, being unrelated to the drug concentration (receptors, ion channels, etc.).

Most of the studies were done on genes encoding for CYP450 enzyme family, the most important drug metabolizing enzyme. Most drug metabolizing enzymes exhibit clinically relevant genetic polymorphisms. The potential of pharmacogenetics in differentiating responders from nonresponders in a patient population with the same diagnosis is promising for its high practical implications, especially for drugs that are substrates of highly

polymorphic enzymes. However, genetic variants linked to receptors have been studied too and represent a powerful direction in predicting drugs response.

## 5.5 Pharmacogenetic profiles

There are two approaches to creating genetic profiles enabling optimal treatment. The first approach implies making a specific hypothesis on the genes that cause therapeutic response modification and their testing in all individuals irrespective of their therapeutic response (gene candidates).

The second approach implies the search for so-called SNP profile (SNP prints) associated with efficient or adverse events in a respective population (forensic precision). This is known as the pharmacogenetic approach, i.e. search for SNP profile. According to literature data reported by experts in the field of pharmacogenetics, for clinical use preference is given to the search for SNP profile in individuals by whole genome scanning. Examples of specific genes modifying drug response, and which could be currently used in clinical practice are the genes encoding for drug metabolizing enzymes from the families CYP450, CYP2D6, 2C19 and 2C9, then phase II enzymes NAT2 and TPMT, B<sub>2</sub>-AR receptors, and some enzymes involved in the metabolism of antitumor drugs.

## 5.6 Guidelines and Recommendations in Pharmacogenetics

Diversity in technology, methodology, genotyping profiles and clinical practice used in pharmacogenetics approach of therapy individualization has inspired the New York National Academy of Clinical Biochemistry Expert Group to develop the Laboratory Medicine Practice Guidelines in Pharmacogenetics (LMPG) Guidelines and Recommendations for Laboratory Analysis and Application of Pharmacogenetics to Clinical Practice. The Expert Committee led by Roland Valdes has issued a draft version of document 60806 open for comments in 2006. The draft version 0606 has been closed for comments, but can still be downloaded as pdf-file from the following web site:

<http://www.aacc.org/AACC/members/nacb/LMPG/OnlineGuide/DraftGuidelines/Pharmacogenetics/>

The objective of LMPG in Pharmacogenetics is to provide a systematic overview of the pharmacogenetics discipline as it applies to clinical laboratory testing and its use in clinical practice. Issues to be addressed refer to methodological (pre-analytical and analytical) consideration, standardization and quality assurance of testing; selection of appropriate pharmacogenetics 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 clinical setting. The framework of LMPG in Pharmacogenetics offers establishing the optimal use of pharmacogenetic information obtained from clinical laboratory testing. It also defines criteria and critical pathways that should be met before testing efficacy is precisely assessed.

The whole project has been divided into nine different sections, each of them chaired by an expert in the field, member of the Committee. The approach of the Committee was to establish a series of questions in each of the sections (outlined below), listed at the beginning of each section followed by a series of respective recommendations and accompanied by a list of most relevant references. The 10th section contains the glossary devoted to pharmacogenetics.

In this paper, the material issued by the New York National Academy of Clinical Biochemistry Expert Group on Laboratory Medicine Practice Guidelines in Pharmacogenetics (LMPG) was

in part taken in its integral form and in part modified for presentation to the Course participants. The participants are encouraged to visit the original document available at the New York National Academy of Clinical Biochemistry web site: <http://www.aacc.org/AACC/members/nacb/LMPG/OnlineGuide/DraftGuidelines/Pharmacogenetics/>

The sections, authors, reviewers and questions are listed below.

1. Valdes R. Guidelines and Recommendations for Laboratory Analysis and Application of Pharmacogenetics to Clinical Practice (<http://www.aacc.org/...>)

In General introduction and scope the author describes the steps in this enormous work on the project as follow:

- 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,
- Define the potential links in the roles of pharmacogenetics and therapeutic drug monitoring in clinical settings,
- Discuss and formulate recommended guidelines for clinical laboratories introducing pharmacogenetics-testing services,
- 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.?, and
- Provide third party payers and regulators of diagnostic laboratory testing recommendations for optimizing their reimbursement and regulatory functions.

2. Ruano G, Valdes R. Pharmacology and population genetics considerations and their applications in pharmacogenetics (Reviewers: Weber WW, DeLeon J) (<http://www.aacc.org/...>)

The 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 pharmacogenetics testing as a discipline. Questions for consideration in this chapter were:

- What are the essential elements of drug pharmacokinetics and pharmacodynamics necessary to understand the application of pharmacogenetics in laboratory medicine?
- What is the cytochrome P450 system and what are the relevant allele frequencies of these components (CYP2D6, CYP2C19, and CYP2C9)?
- What are key considerations and recommendations for statistical sampling of the indicated alleles in populations?

The reader can find sufficient data for understanding drug metabolism, pharmacokinetics and pharmacodynamics as well as the connection between genotyping and drug dosing requirements and adverse drug reaction. The CYP P450 system and special CYP enzymes and

recommendations for their use in pharmacogenetic testing, and how the allele distribution should be evaluated in a service to a reference population are described.

3. Payne D. Methodology and quality assurance considerations in pharmacogenetics testing (Reviewer: Carr J) (<http://www.aacc.org/...>)

The objective of the section is quality assurance and quality control issues. Questions for consideration were:

- What is the error rate for each test and each platform?
- What potential haplotypes, single nucleotide polymorphisms, pseudogenes, epigenetic modifications, or GC ratios could produce inaccurate results for each instrument and/or assay?
- What substances or specimen types could interfere with the various instrument platforms?
- 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?
- 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?
- If software is used in interpretation, will it automatically flag extremely rare or unlikely allele combinations?

Recommendations suggest importance of the evidence on discrepant results from various instruments or within the same instrument, corrective actions, controls of potential enzyme inhibitors in assay, assay validation, etc.

4. Linder M, Steimer W. Clinical laboratory services considerations (Reviewers: O’Kane D, Lyons E) (<http://www.aacc.org/...>)

This section is focused on the importance of how clinical laboratory is expected to provide services of pharmacogenetic testing consistent with the needs of healthcare providers. Questions for consideration were:

- What level of certification should be required for clinical laboratories and personnel performing pharmacogenetics testing?
- What are the recommended specimens for testing?
- What should be the primary test-result output?
- What test result turn-around times are optimal for pharmacogenetics testing?
- What criteria should be used to establish which genetic variants of a locus should be included for diagnostic purposes?
- Is it necessary to have evidence to demonstrate cost effectiveness before recommending clinical use of pharmacogenetic tests?

In this section, the personnel performing pharmacogenetics testing rank first in the recommendation, followed by robust and optimized diagnostic methods for DNA analysis from fresh whole blood, dried whole blood spots, isolated nucleated blood cells, and oral epithelial cells obtained from either buccal scraping or saliva. Laboratories should report a description of all physical characteristics of the genetic locus that are being determined by the

assay. 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 patient's genotype in due time to avoid any risk for the patient.

Before the pharmacogenetics 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 respective 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?

The recommended factors that should be assessed are presented in table below.

	<b>Factors to assess</b>	<b>Features that favor cost effectiveness</b>
Gene	Prevalence Penetrance	Variant allele is relatively common 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 Outcomes and economic impacts	High disease prevalence in the population 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

The use of pharmacogenetics will probably prove most cost efficient in case of drugs with a narrow therapeutic index, high inter-individual variability, problems in monitoring ADR or treatment response, and few alternative therapeutic options.

5. Morello JP, Valdes R. Reporting and interpretation of pharmacogenetic test results (Reviewers: Kirchheiner J, Reynolds D) ([http://www.aacc.org/...](http://www.aacc.org/))

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. Questions for consideration are:

- What information should accompany the reported result?
- Should the result be linked to a specific drug usage (as indicator)? Should drug “dosing and usage” information accompany the test result?
- Should laboratories reporting pharmacogenetic test results have a genetic counseling component or service available or by referral?
- Should manufacturers of IVD used for providing pharmacogenetic tests be required to supply evidence of specific use for every drug, or by class?
- Are there unique or specific limitations to be considered regarding confidential reporting of pharmacogenetic test results?

In recommendation, the authors state that 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. Many other recommendations are counted in consideration of the section.

6. Ruano G, Linder MW. Clinical practice considerations (Reviewers: DeLeon J, Flockhart D) ([http://www.aacc.org/...](http://www.aacc.org/))

This section describes one of the most challenging aspects of transitioning the science of pharmacogenetics to the bedside while establishing criteria for its clinical application. The 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. Three models are presented in which pharmacogenetic test results have proved useful to establish criteria for clinical applications: warfarin (anticoagulation), atomoxetine (psychiatry), and irinotecan (oncology). These three models combined demonstrate several strategies and concepts for future development of pharmacogenetics applications. Questions for consideration were:

- Which are the most current variant alleles for CYP2D6, 2C9, and 2C19 recommended for clinical use?
- What set of criteria (characteristics) should be required of a pharmacogenetic test to make it clinically useful?

- What examples are available that can presently be used as models for application of pharmacogenetic testing in clinical settings? warfarin (2C9 and VKOR), atomoxetine (2D6), irinotecan (UGT1A1)?
- What models of pharmacogenetics-genotyping are available which can serve to establish dosing adjustment rules based on pharmacogenetic information?
- What role should the clinical laboratory play in developing the use of pharmacogenetic testing?

In recommendation, the most current variant alleles are recommended when performing pharmacogenetics-genotyping for CYP2D6, 2C9, and 2C19, as well as its clinical use. As examples, warfarin, atomoxetine and irinotecan are presented.

7. Shaw L, Burckart G. TDM and pharmacogenetics interface considerations (Reviewer: Linder M) (<http://www.aacc.org/...>)

This section addresses medications which require monitoring of their concentrations in blood, i.e. those where narrow therapeutic concentration ranges in blood are required for efficacy, and where toxicity is a persistent problem. As the discipline of pharmacogenetics finds its way into clinical practice, the combination of traditional TDM and pharmacogenetics must be explored to achieve optimum utilization of the combined information they provide. In essence, pharmacogenetics provides information that allows the clinician to make a determination of appropriateness and risk of drug therapy prior to the initiation of therapy. Pharmacogenetics may then have an additional place in selecting drug or dosage alterations during the treatment for a disease process. Questions for consideration are:

- Will use of pharmacogenetics information preclude or require TDM in future? and, How should standard TDM practices be modified to account for pharmacogenetic variation?
- Are there specific clinical situations demonstrative of both TDM and pharmacogenetics information having complementary value?
- How can TDM be best utilized in establishing the predictive value of pharmacogenetic tests, as end point, etc.?

It is recommended that pharmacogenetics testing information be used for the initial selection of drugs or doses for some agents, with a note that the clinician should be aware of the possibility of a significant variability using pharmacogenetics information to design a drug regimen, and that TDM is still essential to monitor therapeutic response and toxicity.

8. Jortani S, McLeod H, Wong S. Ancillary applications (drug prescription /dispensing, forensics) (Reviewer: Wu A) (<http://www.aacc.org/...>)

This section is focused on other applications of pharmacogenetics including its use in: production of targeted drugs by pharmaceutical companies; forensics; safer distribution of medications by pharmacist; environmental toxicology (toxicogenomics); predicting addiction to substances; etc. The information is still rather green, however, evolving rapidly in many of these areas. Yet, in the areas of forensics and drug dispensing some recommendations are worthy of consideration.



## A) Applications in dispensing of medications

Questions for consideration are:

- Should information related to pharmacogenetic test availability be made part of the information provided to patients as part of the drug dispensing mechanism? If so, by whom or how?
- Should pharmacogenetic test information be considered an integral part of the drug-dispensing safety awareness practice?
- Should information-related relationships be fostered between drug dispensing providers and clinical laboratories providing pharmacogenetics testing services?

According to the recommendation, after appropriate consent from the patient, pharmacogenetic genotype information should be made available to drug-dispensing organizations to be used as part of their drug-dispensing safety verification procedures.

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.

## B) Applications in forensics

Questions for consideration are:

- In forensic applications of pharmacogenetics testing, what is (are) the preferred specimen(s), and what diligence should be established for purposes of evidence acquisition?
- What type of information and correlations should be used to optimize the application of pharmacogenetics data in forensic cases?
- What qualifications by way of training and experience should be required for individuals reporting and interpreting pharmacogenetics information when applied to forensics?
- What type of information should accompany a pharmacogenetic test report as it applies to applications in forensics?
- Are there any particular or specific ethical considerations that may apply to the use of pharmacogenetics data with regard to applications in forensics?

According to the recommendation, in forensics blood is considered to be the preferred specimen of choice and should be used whenever available. Chain of custody should be maintained for forensic samples according to the established protocols by each laboratory. 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. Interpretation of pharmacogenetic testing results in forensic toxicology should be done by toxicologists with adequate training in pharmacogenetic testing and familiarity with metabolic pathways.

9. Frueh F, Rahman A. Regulatory considerations (Reviewers: Collins J, Rudman A) (<http://www.aacc.org/...>)

Considering regulatory considerations in this section, the evaluation and approval of pharmacogenomic tests can be categorized into two broad aspects: analytical validation and clinical usefulness of the test. Questions for consideration are:

- When will a test be “required”, when is a test “recommended”?
- When should the label state that the test is “available”?

According to the recommendation, a test may be required for therapy when the drug or the biological is co-developed with a test. Patients are eligible to receive a treatment only if a test result is obtained prior to treatment initiation. 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/biological.

10. Farkas D. Glossary - definition useful in understanding pharmacogenetics. The Glossary is printed as a whole text at the end of this Handbook.

## 5.7 In Conclusion

A hundred years ago, clinicians prescribed a drug only on the basis of physical examination. At the end of the 20<sup>th</sup> century, therapeutic decision was greatly facilitated by laboratory support and the process of therapeutic drug monitoring. Now we have entered a new era with pharmacogenetics and pharmacogenomics, which appear highly promising in enhancing the support to therapeutic decision making, predicting patients who are most likely to respond best to a particular drug, or in whom the drug will yield optimal effects.

The development and release of these LMPG in Pharmacogenetics in terms of methodology, genotyping profiles, interpretation of pharmacogenetic results, quality control and standardization, thereby upgrading the overall healthcare level and service cost effectiveness while reducing the morbidity and mortality rates due to ADR, will ensure appropriate and systematic assessment of pharmacogenetic testing and its optimal application in therapy individualization.

**Recommended literature:**

1. Topić E, Štefanović M. Farmakogenetika. In: Topić E, Primorac D, Janković S, ed. Medicinskobiokemijska dijagnostika u kliničkoj praksi. Medicinska naklada Zagreb, 2004;325-37.
2. Krekels EH, van den Anker JN, Baiardi P, Cella M, Cheng KY, *et al.* **Pharmacogenetics** and paediatric drug development: issues and consequences to labelling and dosing recommendations. *Expert Opinion on Pharmacotherapy* 2007;8(12):1787-99.
3. Koo SH, Lee EJ. Pharmacogenetics approach to therapeutics. *Clinical & Experimental Pharmacology & Physiology* 2006;33(5-6):525-32.
4. Lesko LJ. Personalized medicine: elusive dream or imminent reality?. *Clinical Pharmacology & Therapeutics* 2007;1(6):807-16.
5. McLeod HL. Pharmacogenetic analysis of clinically relevant genetic polymorphisms. *Clinical Infectious Diseases* 2005;41Suppl;7:S449-52.
6. Davies SM. Pharmacogenetics, pharmacogenomics and personalized medicine: are we there yet?. *Hematology*. 2006;2006:111-7.

<http://www.aacc.org/AACC/members/nacb/LMPG/OnlineGuide/DraftGuidelines/Pharmacogenetics/>