Polymorphisms of Phase I enzymes and their role in drug toxicity

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What is Genetic Polymorphism?

A combination of the Greek words poly and morph (multiple and form)

Genetic polymorphism is a difference in DNA sequence among individuals, groups, or populations.
Sources include single nucleotide polymorphisms (SNPs), sequence repeats, insertions, deletions, and recombination.

Drug metabolism

Inter-individual variation of drug effects

Genetic polymorphisms of drug metabolizing enzymes give rise to distinct subgroups in the population that differ in their ability to perform certain drug biotransformation reactions.

Polymorphisms are generated by mutations in the genes for these enzymes, which cause decreased, increased or absent enzyme expression or activity by multiple molecular mechanisms.

The metabolism of drugs and other xenobiotics into more metabolites is essential for their elimination from the body, as well as for termination of their biological and pharmacological activity.

Drug metabolism or biotransformation reactions are classified as;

- **Phase I functionalization reactions:**

oxidation, reduction, hydrolysis.

- Phase II conjugation reactions.

- Both phase I and phase II reactions convert relatively lipid soluble drugs into relatively inactive and more water soluble metabolites, allowing for more efficient systemic elimination.
- The enzyme system involved in the biotransformation of drugs are localized primarily in the **liver**. Other organs with significant metabolic capacity include GI tact, kidney and lung.
- These biotransformation reactions are carried out by CYPs (CytochromeP450 isoforms) and by a variety of transferases.

Genetic differences in drug metabolism are the result of genetically based variation in alleles for genes that code for enzymes responsible for the metabolism of drugs.

In polymorphisms, the genes contain abnormal pairs or multiples or abnormal alleles leading to altered enzyme function.

Differences in enzyme activity occur at different rates according to racial group.

SNPs…. Single Nucleotide Polymorphisms

SNPs are the most common type of genetic variation among people. Each SNP represents a difference in a single nucleotide.

Single changes in one allele of a gene responsible for a variety of metabolic processes including enymatic metabolism.

Variations of SNPs are found in the DNA between genes. They can act as biological markers, helping scientists locate genes that are associated with disease. When SNPs occur within a gene or in a regulatory region near a gene, they may play a more direct role in disease by affecting the gene's function.

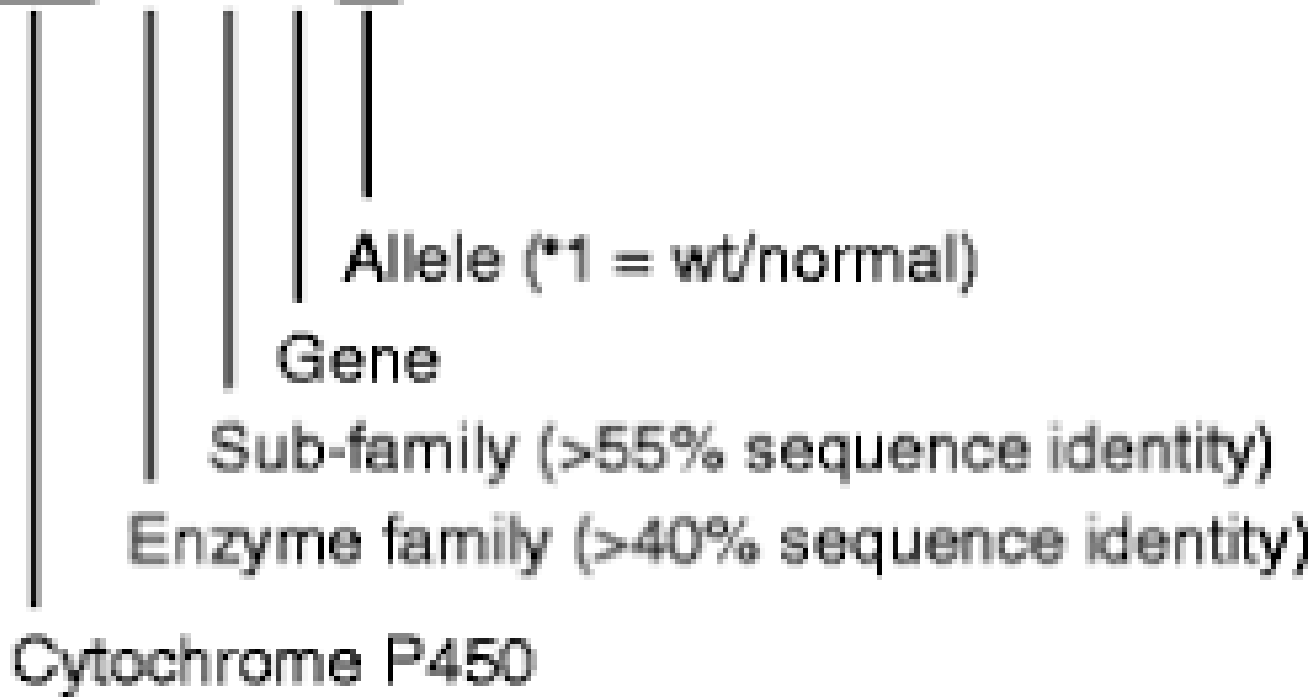
Nomenclature and Classification

Membrane-bound within a cell (cyto) + heme pigment (chrome and P) + absorbs light at 450 nm (450) when exposed to CO.

CYPs are classified according to their amino acid sequence:

1. **Families** proteins with 40% amino acid seq e.g: CYP2, CYP3
2. **Subfamilies** member of family have 55% aminoacid sequence e.g: CYP2D, CYP3A
3. **Individual genes** denoted numerical e.g: CYP2D6, CYP3A4

- Nomenclature: CYP 2 D 6 *4



The Four Metabolizer Types

1. **Poor metabolizer (PM):** Patients who are poor metabolizers experience a very slow breakdown of medications, making side effects more pronounced. That means standard doses of certain medications may not work as intended.
2. **Intermediate metabolizer (IM):** A slowed metabolism may impact breakdown of medications, causing effects similar to poor metabolizers, but not as pronounced.
3. **Extensive (normal) metabolizer (EM):** Considered a "normal" rate of metabolism. Patients are likely to metabolize medication normally and medication is likely to work as intended.
4. **Ultrarapid metabolizer (UM):** Patients in this group metabolize medications too quickly to experience relief from symptoms of depression or other disorders.

Pharmacogenetics

Is the study of how people respond differently to drug therapy based upon their genetic makeup or genes.

Genetic Polymorphisms in Genes that Can Influence Drug Metabolism – CYP450 Isoforms

P450 Enzymes in Drug Metabolism

The polymorphic CYP enzyme superfamily is the most important system involved in the biotransformation of many endogenous and exogenous substances including drugs, toxins, and carcinogens.

Genotyping for CYP polymorphisms provides important genetic information that help to understand the effects of xenobiotics on human body.

For drug metabolism, the most important polymorphisms are those of the genes coding for CYP2C9, CYP2C19, CYP2D6, and CYP3A4/5, which can result in therapeutic failure or severe adverse reactions.

CYP1A1

CYP1A1 plays a major role as a carcinogen activating enzyme within the CYP system. Unlike most CYP enzymes, CYP1A1 expression is mainly found in **extra hepatic tissues**, including the **lung**, where it metabolizes and is markedly induced by **polycyclic aromatic hydrocarbons (PAHs)**.

Elevated CYP1A1 inducibility is associated with pulmonary PAH-related DNA adduction and high lung cancer risk. Both CYP1A1 expression and the formation of these PAH-DNA adducts in human lung tissue are highly variable, possibly due to differing exposure to environmental factors and to genetic polymorphisms affecting the CYP1A1 gene locus .

CYP1A1 substrates include combustion and tobacco products, polycyclic aromatic hydrocarbons (**PAHs**), heterocyclic aromatic amines (HCA) found in charred meat, and industrial arylamines.

CYP1A2

CYP1A2 is one of the CYP450 mixed-function oxidase system that is responsible for the metabolism of xenobiotics in the body and is involved in the synthesis of cholesterol, steroids and other lipids.

In addition, CYP1A2 is an important enzyme that bioactivates a number of procarcinogens including polycyclic aromatic hydrocarbons, heterocyclic aromatic amines/amides, mycotoxins and some natural compounds such as aristolochic acids present in several Chinese herbal medicines.

Furthermore, this enzyme metabolizes a large number of essential endogenous compounds including retinols, melatonin, steroids, uroporphyrinogen and arachidonic acids. In humans, the CYP1A2 enzyme, encoded by the CYP1A2 gene, is of important clinical interest due to the large number of drug interactions associated with its induction and inhibition

CYP1A2 subsrates caffeine, theophylline, clozapine, olanzapine, tizanidine and melatonin.

CYP1A2*1C… alleles are "slow" metabolizers

CYP1A2*1F*…. …. alleles are "fast" metabolizers.

e.g. The same amount of caffeine will therefore tend to have more stimulating effect on CYP1A2 slow metabolizers than on CYP1A2 fast metabolizers.

CYP1A2*1K…. …. Decreased enzyme activity.

CYP2C9

CYP2C9 accounts for approximately 20% of total hepatic CYP content and metabolizes approximately 15% clinically used drugs including S-warfarin, tolbutamide, phenytoin, losartan, diclofenac, and celecoxib. To date, there are at least 33 variants of CYP2C9 (*1B through to *34) being identified.

Warfarin has served as a practical example of how pharmacogenetics can be utilized to achieve maximum efficacy and minimum toxicity. Polymorphisms in CYP2C9 have the potential to affect the toxicity of CYP2C9 drugs with somewhat lower therapeutic indices such as warfarin, phenytoin, and certain antidiabetic drugs. CYP2C9 is one of the clinically significant drug metabolising enzymes that demonstrates genetic variants with significant phenotype and clinical outcomes.

Subsrates of CYP2C9 including drugs with a narrow therapeutic index such as **warfarin** and phenytoin, and other routinely prescribed drugs such as acenocoumarol, tolbutamide, losartan, and some nonsteroidal anti-inflammatory drugs.

Warfarin (Coumadin)

Warfarin, sold under the brand name Coumadin among others, is a medication that is used as an **anticoagulant**.

Polymorphisms in two genes VKORC1 and CYP2C9 play a particularly large role in response to warfarin.

VKORC1 polymorphisms explain 30% of the dose variation between patients.

CYP2C9 polymorphisms explain 10% of the dose variation between patients, mainly among Caucasian patients as these variants are rare in African American and most Asian populations.

Poor Metabolizers…... **Severe bleeding**

CYP2C19

CYP2C19 is an important drug metabolizing enzyme that catalyzes the biotransformation of many other clinically useful drugs including antidepressants, barbiturates, proton pump inhibitors, antimalarial, and antitumor drugs.

1. Dapsone

The adverse effects seen during treatment with dapsone, an antibacterial and antiprotozoal agent, are **hemolysis** and **methemoglobinemia**. The CYP2C19 isoforms are mainly responsible for hemotoxicity of dapsone.

2. Clapidogrel

Clopidogrel, sold under the trade name Plavix among others, is an antiplatelet medication used to reduce the risk of heart disease and stroke in those at high risk. It is also used together with aspirin in heart attacks and following the placement of a coronary artery stent

Several landmark studies have proven the importance of CYP2C19 genotyping in treatment using clopidogrel. In March 2010, the FDA put a black box warning on Plavix to make patients and healthcare providers aware that CYP2C19-poor metabolizers, representing up to 14% of patients, are at high risk of treatment failure and that testing is available. Patients with variants in cytochrome P-450 2C19 (CYP2C19) have lower levels of the active metabolite of clopidogrel, less inhibition of platelets, and a 3.58-times greater risk for major adverse cardiovascular events such as **death, heart attack, and stroke**; the risk was greatest in CYP2C19 poor metabolizers.

CYP2D6

CYP2D6 is most extensively studied polymorphic drug metabolizing enzyme.

Debrisoquin formerly used in the treatment of hypertension, is metabolized by CYP2D6 to 4-hydroxydebrisoquine. Debrisoquine is frequently used for **phenotyping the CYP2D6 enzyme.**

- Impaired ability to hydroxylate, and therefore, inactivate debrisoquin
- Remarkable interindividual variation in pharmacological effect of the drug

Drugs linked to this phenotype should be given in lower doses to PM (PMs… lower urinary concentration, higher plasma concentrations) individuals than EM to reduce risk of overdose and toxic effects.

On the other hand ; Codeine is oxidized to morphine by CYP2D6

- necessary for codeine's analgesic effect
- PMs may have no therapeutic effect

Antidepressants, antiarrhythmics, beta-blockers, and opioid analgesics are typical **substrates of CYP2D6**.

CYP3A4

CYP 3A4 is an important enzyme in the body, mainly found in the liver and in the intestine.

While over 28 single nucleotide polymorphisms (SNPs) have been identified in the CYP3A4 gene, it has been found that this does not translate into significant interindividual variability in vivo. It can be supposed that this may be due to the induction of CYP3A4 on exposure to substrates.

Substrates of CYP3A4

CYP enzymes metabolize approximately 60% of prescribed drugs, with CYP3A4 responsible for about half of this metabolism; substrates include acetaminophen, codeine, cyclosporin, diazepam, erythromycin, some steroids and carcinogens.

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