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9. THE ROLE OF PHARMACOGENETICS IN MANAGEMENT OF CARDIOVASCULAR DISEASE

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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 discomfort to the patient, through serious clinical illness, to the occasional fatality. Recent studies have revealed that 1 in 15 (6.7%) of hospital admissions are due to adverse drug reaction, and 1 in 300 (0.3%) drug reactions has a fatal outcome. This figure ranks adverse drug reactions between the fourth and sixth leading causes of death in hospital patients. The potential risk factors for drug inefficacy or toxicity include biological factors (gender, age, renal and liver function, or other disease factors), lifestyle (smoking, alcohol consumption, diet, drug-drug interaction), and inherited factors.

However, of greater importance are inherited factors that affect the kinetics and dynamics of numerous drugs. Genetic variation in genes for drug-metabolising enzymes, drug receptors, and drug transporters has been associated with individual variability in the efficacy and toxicity of drugs. There is a relationship between genetic predisposition of an individual and his ability to metabolise a drug. Differences in drug metabolism can lead to severe toxicity or therapeutic failure due to a change in the ratio between the drug dose and the concentration of pharmacologically active substance in the blood, as the result of genetic modifications. Genetic polymorphism based on the ability to metabolise the drug is associated with three phenotype classes: -

- The phenotype of the extensive drug metabolizer (EM) is characteristic of the normal population. EM alleles behave in a dominant way. Individuals with EM phenotype are either homozygous or heterozygous for the wild-type allele.
- The poor metabolizer phenotype (PM) is associated with the accumulation of specific drug substrates in the body, and is inherited as a recessive autosomal trait 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 ultra-extensive metabolizer phenotype (UEM) is characterized by enhanced drug metabolism due to gene amplification inherited as an autosomal dominant trait. Individuals with the ultrarapid phenotype are prone to therapeutic failure because the drug concentrations in the plasma at normal doses are by far too low.

5-20% of patients can belong to one of these risk groups, depending on the population studied.

Most of the works on this topic have concentrated on the cytochrome P450 enzyme (CYP), which is a highly polymorphic enzyme that plays a key role in metabolising the majority of drugs in the human body.

9.1 The cytochrome P450 superfamily

The superfamily of cytochrome P450 (CYP450) enzymes is the most important oxidative enzymatic system involved in drug metabolism. More than forty isoenzymes that show variable catalytic activity and uniform action have been discovered in human subjects to date. Although many of these enzymes exhibit genetic polymorphism, the molecular basis of the modification has only been identified for some of them. The percentage of individual isoenzymes in the human liver greatly varies (Figure 1).

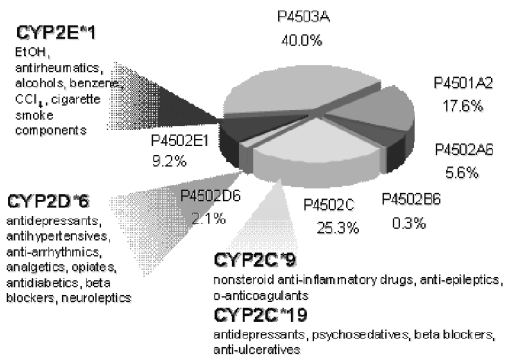

Figure 1: CYP450 enzymes

Table 1. Inducers and inhibitors of major CYP enzymes

Enzyme; substrate	Enzyme inducers	Enzyme inhibitions
CYP1A2		
TCAs	Omeprazole, lansoprazole	Fluvoxamine (other SSRIs weak)
Haloperidol, olanzapine	Phenobarbital, phenytoin, carbamazepine	Ciprofloxacin (other quinolones weak)
Propranolol, local anesthetics	Erythromycin, clarithromycin, rifampin	Cimetidine
Theophylline, caffeine	Cigarette smoke	Isoniazid
Diazepam, chlordiazepoxide	Ritonavir	Oral contraceptives
Estrogens, tamoxifen	Insulin	ticlopidine
CYP2C9		
ASA and most NSAIDs	Rifampin	Fluvoxamine (other SSRIs weak)
Phenobarbital, phenytoin	Phenobarbital, phenytoin, carbamazepine	Amiodarone
S-warfarin, dicumarol		Omeprazole
Losartan (activation)		Ritonavir
Tolbutamide, sulfonamides, dapsone		HMG-CoA reductase inhibitors
Zidovudine		Tolbutamide
Diazepam, temazepam		Cimetidine (weak)
Fluoxetine, meclobemide		Azole antifungals (weak)
CYP2C19		
TCAs	Rifampin	Fluoxetine, fluvoxamine, paroxetine
Diazepam, temazepam	Phenobarbital, phenytoin, carbamazepine	Omeprazole, lansoprazole
Omeprazole, lansoprazole	Prednisone	Ritonavir
Propranolol	Noethindrone	Azole antifungals (weak)
Phenytoin, barbiturates, valproic acid		Cimetidine (weak)
Zidovudine		Ticlopidine
CYP2D6		
TCAs, SSRIs, venlafaxine		Quinidine
Phenothiazines, haloperidol		Fluoxetine, paroxetine, sertraline
Several ß-blockers		TCAs, venlafaxine
Codeine, oxycodone, hydrocodone		Phenothiazines, haloperidol, nefazodone
Dextromethorphan		Ketoconazole
Omeprazole		Cimetidine
Halothane		Ritonavir
MDMA (ectasy)		HMG-CoA reductase inhibitors
Encainide, flecainide, propafenone		Amiodarone, encainide
Selegiline		Chlorpheniramine
CYP2E1		
Acetaminophen	Ethanol	Disulfiram
Ethanol and other alcohols	Isoniazid	Ethanol
Inhalational anesthetics	Clofibrate	Cimetidine
Sulfonamides, dapsone		Isoniazid
CYP3A4		
Halothane	Phenytoin, barbiturates	Ketoconazole, itraconazole, fluconazole

A majority of them belong to the group of family 3A and 2C isoenzymes, followed by family 1A and 2E isoenzymes. Of the 26 mammalian subfamilies, the CYP2C, CYP2D and CYP3A subfamilies are involved in the metabolism of most clinically relevant drugs. It should be noted, however, that it is only relevant in case of drugs which are substrates exclusively or mostly for this particular enzyme. Important substrates, inducers and inhibitors of the major CYP enzymes are listed in Table 1.

9.1.1 CYP2D6

The CYP2D6 isoenzyme is by far most extensively characterized enzyme from the CYP450 superfamily which exhibits a polymorphic expression in humans. It accounts for not more than 2.6% of CYP450 in the liver, and plays a very important role in the metabolism of almost 100 most commonly used drugs. The CYP2D6 locus has been identified as an array of at least three highly homologous genes, on the long arm of chromosome 22. In addition to the active CYP2D6 gene, consisting of nine exons and eight introns, an inactive pseudogene, CYP2D8P, and an inactive homologue, CYP2D7, are present in a tandem array.

Greater than 100-fold variability in CYP2D6 activity has been observed within the general population and can be attributed to genetic polymorphism of the CYP2D6 gene, as the result of multiple mechanisms including single point mutations, insertions or deletions, and major rearrangements such as complete gene deletion, gene duplication or multiplication. Genetic polymorphism of the CYP2D6 locus, its functional nucleotide changes, and structural effects, and occurrence in study subjects are presented in Table 2.

The major genetic polymorphisms associated with the PM phenotype lead to the complete lack of functional protein.

- The most common allele among PMs is CYP2D6*4. This allele results from a single-point mutation that alters messenger RNA (mRNA) splicing, resulting in failure to produce an active protein. The CYP2D6*4 allele accounts for more than 70% of CYP2D6 alleles in PM subjects.
- The second most common allele associated with PM phenotype is the CYP2D6*5 allele (25%), which represents complete deletion of CYP2D6.
- The third most common allele among PMs is the CYP2D6*3 allele (3%). CYP2D6*3 results from deletion of a single nucleotide within the coding region, leading to a frameshift.

The earliest evidence of polymorphic expression was obtained during clinical trials of the antihypertensive debrisoquin, since when the activity of debrisoquin hydroxylase (CYP2D6) has been taken as a standard for the phenotypic analysis of this polymorphism in clinical trials.

As the CYP2D6 polymorphisms are recessive traits, the heterozygous individuals with one active allele and one mutant allele have a metabolism, which does not overtly differ from a person with normal phenotype. However, these heterozygous individuals may have a slightly increased metabolic ratio that points to a deficiency of or reduction in the metabolic capacity. The individuals with these genotypes are referred to as EM and represent a great majority of the population.

Designation	Nature of mutation	Allelic frequency (%)
CYP2D6 *2	$G_{1749}C$, silent mutation, $C_{2938}T$, amino acid change, multiple copies result in increased activity	3.5
CYP2D6 *3	A_{2637} deletion in exon 5	2.7
CYP2D6 *4	$G_{1934}A$, splice side defect, no activity	28.6
CYP2D6 *5	CYP2D6 gene deletion	11.6
CYP2D6 *6	T_{1795}, deletion, premature stop codon	1.8
CYP2D6 *7	$A_{3023}C$, amino acid change	1.5
CYP2D6 *8	$G_{1846}T$	1
CYP2D6 *9	3 bp deletion, loss of K_{281}	<1.5
CYP2D6 *10	$C_{188}T$, $G_{4268}T$; amino acid substitution-decreased catalytic activity	<0.5
CYP2D6 *11	$G_{971}C$, splice defect, no activity	<1

Table 2. CYP2D6 genetic polymorphism

However, studies have shown that the metabolic ratios in heterozygous individuals with multiple mutant alleles approach the ratio seen in homozygous mutant individuals. The metabolic ratio differences indicate that the relative concentrations of 'parenteral' unchanged drug in homozygous individuals can differ by more than five-fold compared with heterozygous individuals, thus higher plasma concentrations of CYP2D6 substrates can be expected in heterozygous individuals with multiple allelic mutations. The fact that there may be 35% of persons with a heterozygous genotype could have a considerable impact on the prevalence of side effects of the CYP2D6 substrates.

The individuals who have inherited two inactive alleles exhibit the poor metabolizer (PM) phenotype. They show complete absence of CYP2D6 activity and impaired ability of metabolising 2D6 substrates.

The ultraextensive (UEM) phenotype results from duplication or amplification of CYP2D6*2 allele on chromosome 22. The most frequent gene duplication results in two copies of the CYP2D6*2 gene on a single chromosome (CYP2D6*2x2). However, even 12- to 13-fold amplification of the gene has been observed. The amplified gene product has functionally the same but catalytically increased activity, which directly correlates with the amount of the same gene in the subjects with this allele. This unique allele has only been identified in the heterozygous form and results in excessive expression of CYP2D6 enzyme. This excessive expression exerts a dramatic effect on the metabolism and clearance of CYP2D6 substrates, and results in sub-therapeutic drug concentrations at a standard dosage. Therefore, the individuals with UEM phenotype may require 'megadoses' of particular drugs (especially those metabolised into inactive metabolites) to achieve therapeutic efficacy.

Significant ethnic variations have been recorded in the prevalence of the CYP2D6 PM phenotype. For instance, in Caucasian populations of Europe and North American, the prevalence of PM phenotype ranges between 5% and 10%. In the Croatian population, the prevalence of PM phenotype relative to the metabolising ability of 2D6 isoenzyme in a small group of healthy volunteers was found to be 3.7%. The prevalence of PM phenotype is 1.8% in Black Americans, 1.2% in the Thai, 1.0% in the Chinese, 2.1% in the authentic Malayan population, and appears to be absent in the Japanese. In Caucasians, the PM phenotype is a consequence of various mutant CYP2D6 genotypes. Thus, 32% of the Caucasian population are heterozygous for the inactivating mutant CYP2D6 allele, so they may exhibit the intermediary phenotype which results from 2D6*4 and 2D6*3 alleles in almost 95% of cases. The prevalence of UEM phenotype in Caucasians is 7%, and results from CYP2D6*2 allele in more than 95% of cases.

CYP2D6 is a very polymorphic enzyme that catalyses the metabolism of about 100 most common drugs such as antiarrhythmics, antidepressants, antipsychotics, opioids, and so on. From the clinical point of view, the common therapeutic dose of antiarrhythmics in these people will produce a proarrhythmic effect or some other toxic effect. Treatment with antidepressants will result in toxicity in PM but will prove inefficient in UEM. In PM patients treated with antipsychotics, tardive dyskinesia is observed. Also opioid drugs in PM may result in inefficacy of codeine as analgesic, narcotic side effects, and eventually in dependency.

9.1.2 CYP2C19

The second best characterized CYP polymorphism is the polymorphism of CYP2C19 isoenzyme, which is involved in the metabolism of a number of drugs, including certain barbiturates, diazepam, mephenytoin, omeprazole, proguanil and propanolol. Individuals can be characterized as either extensive or poor metabolizers. The prevalence of PMs characterized by a reduced drug metabolising ability by CYP2C19 enzyme, inherited as an autosomal recessive trait, has been reported to be 2%-5% in Caucasians, 4%-8% in Africans, 11%-23% in Orientals, and as high as 70% in the residents of Vanuatu in Melanesia.

To date, more than 10 allelic variants of CYP2C19 have been described (Table 3). CYP2C19*2 and CYP2C19*3 have been shown to be the main mutations contributing to PM phenotype.

Allele	Trivial name	Effects of nucleotide changes	Enzyme activity
CYP2C19*1A	$CYP2C19_{wt1}$		Active
CYP2C19*1B	$CYP2C19_{wt2}$	$Ile_{331}Val$	Active
CYP2C19*2A	$CYP2C19_{m1A}$	Splicing defect	Inactive
CYP2C19*2B	$CYP2C19_{m1B}$	$Glu_{92}Asp$ splicing defect	Inactive
CYP2C19*3	$CYP2C19_{m2}$	Stop codon	Inactive
CYP2C19*4	$CYP2C19_{m3}$	GTG initiation codon	Inactive
CYP2C19*5A	$CYP2C19_{m4}$ $CYP2C19_{TRP433}$	$Arg_{433}TRP$	Inactive
CYP2C19*5B		$Arg_{433}TRP$	Inactive
CYP2C19*6	$CYP2C19_{m5}$	$Arg_{132}Gln$	2% rel. activity
CYP2C19*7	$CYP2C19_{m6}$	Splicing defect $G_1T_2 \rightarrow G_1A_2$	
CYP2C19*8	$CYP2C19_{m7}$	$Trp_{120}Arg$	9% rel. activity

Table 3. Allelic variation of the CYP2C19 gene

The main genetic impairment in PM phenotypes is the consequence of mutant CYP2C19*2 allele, arising from single G®A mutation in exon 5, resulting in the formation of a new, unnaturally spliced CYP2C19 mRNA. Translation of this mRNA transcribes the formation of inactive protein. This mutation is found in about 75% - 83% of Caucasians and Orientals genotyped as PMs. The other mutant allele, designated as CYP2C19*3, arises from G®A transition at position 636 in exon 4 of CYP2C19, which results in a premature stop codon and produces a truncated protein. This allele is rare in Caucasians but appears to account for the remaining defective alleles in Oriental PMs. Clinical implications of the CYP2C19 polymorphism have not yet been thoroughly described. In the light of the relative percentage of this isoenzyme and identification of an even greater number of pharmaceutical substrates metabolised by CYP2C19, the clinical relevance of this polymorphism will hopefully be elucidated in the near future.

9.1.3 CYP2C9

CYP2C9 is a polymorphic enzyme of the CYP450 superfamily that catalyses the metabolism of well-known drugs such as S-warfarin, tolbutamide, phenytoin, glipizide, losartan, and many non-steroidal anti-inflammatory drugs. The drug effects may include hemorrhage, hypoglycaemia, phenytoin toxicity, and decreased antihypertensive effect in patients phenotyped as poor metabolizers. Bleeding is the major complication of warfarin therapy. At present, at least six different CYP2C9 alleles have been found in Caucasian population encoding CYP2C9 enzymes with potentially different catalytic activity (Table 4).

Numerous reports concerning CYP2C9*2 and *3 alleles suggest that the catalytic activity of the corresponding enzyme is reduced compared with the wild type. Individuals possessing at least one variant allele exhibit significant reductions of 85%-95% in the CYP2C9 metabolic activity and consequently in dosing requirements of certain CYP2C9 substrates. Caucasians appear to carry 8%-20% of CYP2C9*2, and 6%-10% of CYP2C9*3 variants, whereas the respective figures in Asians range between 0% and 2-5% only.

9.1.4 CYP2E1

The CYP2E1 isoenzyme is responsible for the metabolism and bioactivation of many pro-carcinogens and some drugs, and is a major alternative system of metabolising ethanol. CYP2E1 is encoded by a gene located on chromosome 10, with seven different loci recognized to date. Two alleles of the gene have been identified, i.e. C and c2. The mutant c2 allele lacking the RsaI restriction site is associated with a higher translation activity, protein level, and enzyme activity. The locus polymorphism, related to modifications of restriction cleavage by Rsa I and Pst I endonucleases, exhibits a 10-fold increase in the gene transcription activity. The absence of the rare C allele has been associated with lung cancer in the Japanese, whereas no such association has been observed in Caucasian and African Americans in the USA.

The expression of CYP2E1 mRNA is increased in the individuals who are homozygous or heterozygous for the c2 mutant CYP2E1 allele. Thus, this mutation may potentially lead to an increased functional protein

Allele	Effects of nucleotide changes	Enzyme activity	Allelic frequency (%)	
			Caucasians	Orientals
CYP2C9*1	Wild type	Active	~ 70	~ 95
CYP2C9*2	$C_{430}>T \rightarrow Arg_{144}Cys$	Decreased activity	8-20	0
CYP2C9*3	$A_{1061}>C \rightarrow Ile_{359}Leu$	Decreased activity	6-10	2-5
CYP2C9*4	$T_{1076}>C \rightarrow Ile_{350}Thr$	?	?	?
CYP2C9*5	$C_{1080}>G$ $Asp_{360}Glu$	?	0	~3
CYP2C9*6	A_{818}, base deletion	?	?	?

Table 4. Allelic variation of the CYP2C9 gene

The most common allele is designated as CYP2C9*1 allele, and is considered the wild type allele. CYP2C9*2 allele has a point mutation (C430®T) in exon 3 causing an Arg144Cys amino acid exchange, whereas the point mutation (A1061®C) in exon 7 of CYP2C9*3 results in Ile359Leu exchange. Recently, another substitutions at base pair 1076 T®C, encoding for an Ile359Thr (CYP2C9*4), and 1080 C®G, encoding for an Asp360Glu amino acid substitution (CYP2C9*5) have been identified. Finally, a new null polymorphism, CYP2C9*6, containing an adenine base pair deletion at nucleotide 818 has been identified, resulting in a premature stop codon and a truncated, inactive protein.

expression, and result in enhanced metabolism of CYP2E1 substrates. Although there is evidence for the presence of the CYP2E1 genetic polymorphism in humans, the exact molecular basis and its clinical significance have not yet been established.

9.1.5 CYP3A

The CYP3A subfamily includes 3A4 and 3A5 isoenzymes in adults, and 3A7 isoenzyme in fetal liver. The predominating 3A isoenzyme in the liver of adults is 3A4, which accounts for 20%-40% of total hepatic P450 cytochrome. It is also widely expressed throughout the gastrointestinal

tract, kidneys and lungs. Although a high inter-individual variability of the CYP3A4 isoenzyme expression (>20-fold) has been demonstrated in the human liver, the genetic basis of this polymorphic expression has not been established. More than 150 drugs are known substrates of CYP3A4, including many opiate analgesics, steroids, antiarrhythmic agents, tricyclic antidepressants, calcium channel blockers, and macrolide antibiotics. The genetic variability leading to expression or change in the activity of CYP3A4 isoenzyme requires in-depth studies.

9.2 The drug-metabolizing enzymes in cardiovascular pharmacology

Some of the drugs used in cardiovascular pharmacology and metabolised by polymorphic CYP450 enzymes are presented in Table 5.

Drug	Enzyme	Consequences
Losartan	CYP2C9	Not documented
Flecanaide	CYP2D6	Inconsistent ß-blockade
Benzodiazepines	CYP2C19 & CYP3A4	Not documented
Carvedilol	CYP2D6	Variations in a1/ß2 blockades
Metoprolol	CYP2D6	Variations in ß- blocker activity
Warfarin	CYP2C9	Variations in anticoagulant effect

Table 5. Polymorphic drug-metabolising enzymes in cardiovascular pharmacology

9.2.1 Antiarrhythmics

(RS)-propafenone is a widely used class 1C antiarrhythmic drug, which is hydroxylated by CYP2D6 to 5-hydroxypropafenone. The drug is both a ß-blocker and sodium channel blocker. The ß-blocking activity of (RS)-propafenone is mostly due to S-enantiomer, whereas the sodium channel blocking activity is due to both R and S enantiomers. The metabolic product, 5-hydroxypropafenone, has different pharmacologic activity from propafenone, and is mainly a sodium channel blocker with minor ß-adrenergic antagonist effect. Genotyping has shown that poor metabolizers have more ß-blockade and severe side effects when compared with extensive metabolizers. Consequently, another drug that is as effective and less toxic might be considered for these patients.

Flecainide is another class 1C antiarrhythmic drug, which is also metabolised by CYP2D6 but its metabolic products are inactive. The dose recommendations are only valid for the dose range, taking into account individual differences in the metabolising activity due to genetic polymorphism.

9.2.2 Beta blockers

Carvedilol, a ß-blocker with a-adrenergic-blocking effects, metabolised by CYP2D6 and CYP2C9, is a racemic drug whose a1 blocking activity is mainly supported by the (S)-enantiomer, while the ß1-blocking activity is a property of the two enantiomers. The (R)-enantiomer is better metabolised by CYP2D6, which explains the differences in a1/ß1 relative activities, depending on the genotype and expressed alleles.

Metoprolol is another ß-blocker with ß-adrenergic-blocking effect, and is also metabolised by CYP2D6. Thus the treatment has to be adjusted according to the patient's responsiveness.

9.2.3 Anticoagulants

Warfarin is an oral anticoagulant mostly used in patients with venous and arterial thromboembolism. To keep the WHO International Normalised Ratio (INR) values between 2.0 and 3.0, the doses of warfarin fluctuate from 1 mg to 20 or more mg daily. Standardized induction schemes with monitoring of INR over the first 4 days have shown a modest 69% success rate. Such inter-individual variations are partly due to the presence of polymorphisms of the CYP2C9 gene. Warfarin is a racemic drug with a 3- to 5-times higher anticoagulant effect of (S)-enantiomer than of the (R) form. CYP2C9, a CYP450 enzyme responsible for the metabolism of S-warfarin, converts S-warfarin into inactive 6-hydroxy and 7-hydroxy metabolites. The CYP2C9*2 variant is about 12% and CYP2C9*3 is less than 5% as efficient as the wild type enzyme. Up to 20% of the general population could be carriers of the mutant allelic forms and behave as poor metabolizers. Detail genotyping have shown strong correlation between the need of a low dose of warfarin and the presence of at least one mutant allele of CYP2C9 gene. These subjects have difficulties at the time of induction of anticoagulation as indicated by longer hospitalisation or longer visiting outpatient clinics. The decreased metabolism of warfarin also makes carriers four times more susceptible to bleeding complications in spite of low dose administration. The concern for this complication is justified by the high mortality risk, which is increased in the elderly population. Therefore, identification of these polymorphisms before starting therapy should help prevent complications.

9.2.4 Toxic-metabolizing enzyme and risk factors

An interesting application of research on genetic polymorphisms refers to the toxic-metabolising enzymes, which detoxify alcohol and tobacco products, two well-identified risk factors in cardiology.

9.2.5 Alcohol

Moderate alcohol consumption is consistently associated with reduced risk of myocardial infarction (the so-called 'French paradox'), nevertheless, it is not clear whether the apparent benefit of alcohol is due to constituents of the alcoholic beverages other than ethanol or in fact reflects the lifestyle factor. Alcohol dehydrogenase (ADH) izoenzymes are also drug metabolizing enzymes that oxidize ethanol and play a major detoxification role upon alcohol consumption. Although several ADH izogenes exist (ADH1, 2, 3), ADH2 and ADH3 are the only locuses that give different alleles with different kinetic properties (Table 6). Its pharmacokinetics shows that the ?1?1 homodimer is associated with a fast rate of oxidation as compared to ?2?2. Homozygosity for ?2?2 is associated with the highest level of plasma HDL and reduced risk of myocardial infarction as compared to homozygosity for ?1?1.

Class	Gene locus	Protein product	Amino-acid change	Frequency in Caucasians	Enzyme activity
ADH2	ADH2*1	β?	wt	95%	low Km, low V_{max}
	ADH2*2	β_2	Arg47Hys	0-5%	low Km, high V_{max}
	ADH2*3	β_3	Arg369Cys	<1%	high Km, low V_{max}
ADH3	ADH3*1	γ?	Arg271, Ile349	48-62%	low Km, higher V_{max}
	ADH3*2	γ_2	Gln271, Val349	37%	low Km, lower V_{max}

Table 6. ADH genotypes

9.2.6 Tobacco

Glutathione S transferase (GST) belongs to the drug-metabolizing enzymes that detoxify or activate several drugs as well as chemicals in cigarette smoke. GST can eliminate the products of oxidative stress and also carcinogenic compounds. The gene exists in two main forms, GST M1 and GST T1, with different capacity to detoxify tobacco compounds. Some case cohort studies in coronary patients have indicated that ever-smokers with GST M1-0 were at a nearly 2-fold coronary risk compared with that found in ever-smokers with GST M1-1 or never-smokers with GST M1-0.

9.2.7 Lipid lowering drugs

Lipid lowering drugs such as statins are widely used to reduce cholesterol and triglyceride levels, as they may prevent the development of ischemic events in patients with dyslipidaemia or cardiovascular disease. All statins lower cholesterol level through reversible and competitive inhibition of HMG-CoA reductase, an enzyme involved in the biosynthesis of cholesterol and other sterols. This mechanism leads to LDL cholesterol reduction, triglyceride reduction, and HDL cholesterol elevation. The synthetic statins atorvastatin, cerivastatin and fluvastatin appear to be as efficacious as the natural ones such as lovastatin, pravastatin, and simvastatin. Pharmacokinetic properties of statins, listed in Table 7, may suffice to affect the choice of one statins over another in a given clinical situation.

Except for pravastatin, all statins are subject to extensive first-pass metabolism by the CYP450 enzyme. Atorvastatin, lovastatin and simvastatin are metabolised by CYP3A4, whereas fluvastatin is metabolised by CYP2C9. Cerivastatin is jointly metabolised by CYP3A4 and CYP2C8. Pravastatin does not undergo CYP450 metabolism. Statins metabolised by a common enzymatic system may differ in the extent of metabolism by this system, so that each statin has a different potential for drug actions mediated through mutation in CYP450 genes. A study conducted in healthy volunteers to measure the catalytic activity of CYP2D6 polymorphism has revealed that hepatic metabolism of simvastatin is inhibited by the presence of the variant allele of the CYP2D6 polymorphism. In homozygous carriers, poor metabolizers (who represent 3% to 10% of the Caucasian population), a given dose of simvastatin has a higher cholesterol-lowering effect compared with the wild type carriers. In contrast, carriers of the duplicated or multiplicated wild type CYP2D6 gene, called ultrarapid metabolizers, need higher doses of simvastatin to reduce cholesterol levels to the same extent. Knowing in advance the genetic characteristics of the patients might help establish the right doses to be prescribed.

9.3 Future promises

Genetic heterogeneity appears to be a significant source of variability observed in the response to drugs. The process to enhance the efficacy of administered drugs is very old. A hundred years ago, the clinicians prescribed the drug only on the basis of physical examination. At the end of the 20th 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

Characteristic	Atovarstatin	Cerivastatin	Fluvastatin	Lovastatin	Pravastatin	Simvastatin
Prodrug absorption	No	No	No	Yes	No	Yes
(%)	Rapid	No data	98	30	35	60-85
Bioavailability (F)						
(%)	12	60	24	<5	17	<5
Excretion (%)						
Urine	<2	24	5	10	20	13
Feces	>98	70	90	83	70	60
Half-life (hours)	14 (parent)	2-3	<1	3-4	1.8	3
Protein binding (%)	≥90	>99	98	>95	50	95
CYP substrate	CYP3A4	CYP3A4 CYP2C8	CYP2C9	CYP3A4	Sulfation	CYP3A4
Major metabolites contributing to lipid-lowering effects	Yes	Yes	No	Yes	No	Yes
Lipophilicity	Lipophilic	Lipophilic	Hydrophilic	Lipophilic	Hydrophilic	Lipophilic

Table 7. Difference between statins

to respond best to a particular drug or in whom the drug will yield optimal effects. An individual's polymorphism and possible response to a particular drug can be assessed by a dual approach, i.e. by phenotyping or genotyping.

9.4 Phenotyping/genotyping

Phenotyping includes administration of the respective drug (its metabolism being known to exclusively depend on a specific enzyme function), followed by determination of the metabolic ratio (MR), defined as a ratio of drug dose or unchanged drug and its metabolites measured in serum or urine. Determination of the phenotype relative to the reference substrate allows for prediction of phenotype for other substrates. Genotyping includes identification of specific gene mutations that result in a specific phenotype. These mutations include gene mutations that lead to over-expression (gene duplication), absence of active protein product (null allele), or formation of a mutant protein with a decreased catalytic activity (inactivating allele).

In pharmacokinetic studies, preference is given to phenotyping over genotyping for detecting drug-drug interactions or impairments in the overall process of drug metabolism. However, the phenotyping approach also suffers from a number of serious drawbacks, e.g., complex testing protocols, risk of undesired drug reactions, problems of erroneous phenotype allocation due to co-administration of several drugs or possible confounding disease effects. The methods of genotyping, on the other hand, require small amounts of blood or tissue, they are not influenced by either underlying disease or currently used drugs, and results are available within 48-72 hours, thus allowing for prompt intervention.

9.5 Methods of genotyping

A number of molecular biochemistry methods used for recognition of genetic mutations are currently available (Table 8). Almost all of these methods are based on the technique of the respective DNA fragment amplification, for which Mullis was awarded Nobel Prize in 1993. Chain reaction of DNA synthesis by DNA polymerase or simply polymerase chain reaction (PCR) is a relatively new method of nucleic acid synthesis *in vitro*, by use of which a specific DNA fragment can be multiplied in a large number of copies. DNA is isolated from EDTA blood, and

Known polymorphisms		Unknown polymorphisms	
PCR-based amplification		*PCR-based amplification*	
1	RFLP	2	SSCP
3	SSCP	4	DGGE
5	Allele-specific PCR amplification	6	RFLP
7	Multiplex allele specific PCR	8	CCM
9	OLA	10	DS
11	TaqMan	12	HA
13	DNA chip array technique	*Microsequencing*	
Microsequencing			

Table 8. Methods of

a specific region of the gene under study is being amplified by oligonucleotide DNA fragments (primers) complementary with the borderline segments of the known DNA to be amplified (target DNA).

The product of PCR amplification is then submitted to subsequent reactions to identify a known or unknown mutation and/or deletion (RFLP, SSCP, multiplex PCR, allele-specific PCR, etc.) (Figure 2).

for two denatured single strand molecules (ssDNA) to acquire different spatial conformations. Due to these variabilities, characteristic distributions of single strand bands can be discriminated in a high resolution gel, depending on the primary DNA sequence and, accordingly, on the respective genotype. Because of its simple performance, SSCP is mostly employed on screening for known as well as new mutations.

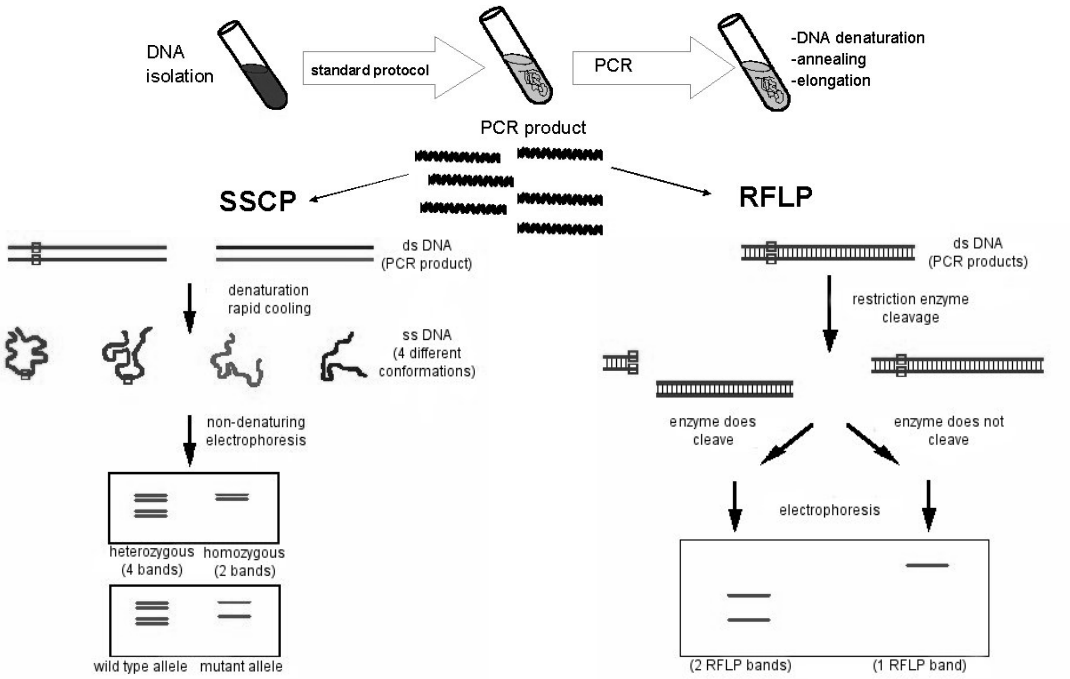

Figure 2. Basic principles of SSCP and RFLP methods

9.5.1 PCR-RFLP

The method of restriction fragment length polymorphism (RFLP) is based on specific PCR product cleavage by restriction endonucleases capable of cleavage of double-stranded DNA with a high level of specificity for nucleotide sequences. The size of digestion products is assessed by agarose gel electrophoresis, staining with ethidium bromide, and UV transillumination. Differences in the size of DNA fragments produced as the result of digestion with restriction endonucleases are known as the restriction fragment length polymorphism. Comparison of the PCR product restriction fragment size between a control and study subject allows for recognition of the subject's genetic mutation and determination of the subject's genotype and phenotype.

9.5.2 PCR-SSCP

Upon genomic DNA amplification, the PCR product is being denatured, yielding single strand DNA fragments. These single strand fragments acquire varying conformation in space, depending on their primary nucleotide sequence. A single nucleotide difference is quite enough

9.5.3 Multiplex allele specific PCR amplification

Multiplex PCR is a method which allows for simultaneous detection of all known polymorphic genes that result in a PM or EM phenotype. First, another DNA fragment including complete CYP2D6 gene is enzymatically amplified by PCR from genomic DNA. Then, two PCR reactions follow from this DNA fragment: one with primers specific for the wild type, and the other with primers specific for the mutant type. The conclusion on the subject's homo-/heterozygosity is derived from these two reactions. Upon PCR product electrophoresis, the genotype of each sample can be directly read according to the occurrence or absence of particular DNA bands in the gel. For a very large number of samples, some of the options available can be chosen, e.g., oligonucleotide ligation assay (OLA), TaqMan allelic discrimination assay (TaqMan), microsequencing, and chip or microarrays techniques, which enable simultaneous genotyping of hundreds to tens of thousands of samples.

	Fragment size (bp)	Sensitivity (%)	Mutation position
SSCP	250	80	No
DGGE	600	95	No
Sequencing	200-400	100	Yes
RFLP	Tens of thousands	<50	No
CCM	1700	>95	Yes
PCR DS	500	>99	Yes
HA	300	80	No

SSCP = single strand conformation polymorphism, DGGE = denaturing gradient gel electrophoresis, RFLP = restriction fragment length polymorphism, CCM = chemical cleavage of mismatch, PCR DS = PCR direct sequencing, HA = heteroduplex analysis
Table 9. Advantages and shortcomings of genotyping methods

These highly efficient techniques are mostly used for genotyping of known polymorphisms. Apart from SSCP and microsequencing, for identification of new, as yet unknown polymorphisms, other methods are also used, e.g., denaturing gradient gel electrophoresis (DGGE), heteroduplex analysis (HA), PCR direct sequencing (PCR-DS), and chemical mismatch cleavage (CMC).

The usability of the methods presently available in molecular diagnosis depends on many criteria, according to which each of the methods has certain advantages as well as shortcomings (Table 9). It is therefore of paramount importance to find proper balance in order to obtain an optimal response at the lowest level of limitations. The method of SSCP is most commonly employed for clinical purpose at molecular biochemistry laboratories for detection of particular polymorphisms, because of its simple performance, rapidity and cost effectiveness, whereas the multiplex allele specific PCR is preferred for simultaneous detection of a number of mutations.

9.6 Clinical use of pharmacogenetics

There is strong evidence for a significant role of the genetic polymorphism of the enzymes involved in the metabolism of drugs in the occurrence of adverse effects. Determination of these genetic variations is highly

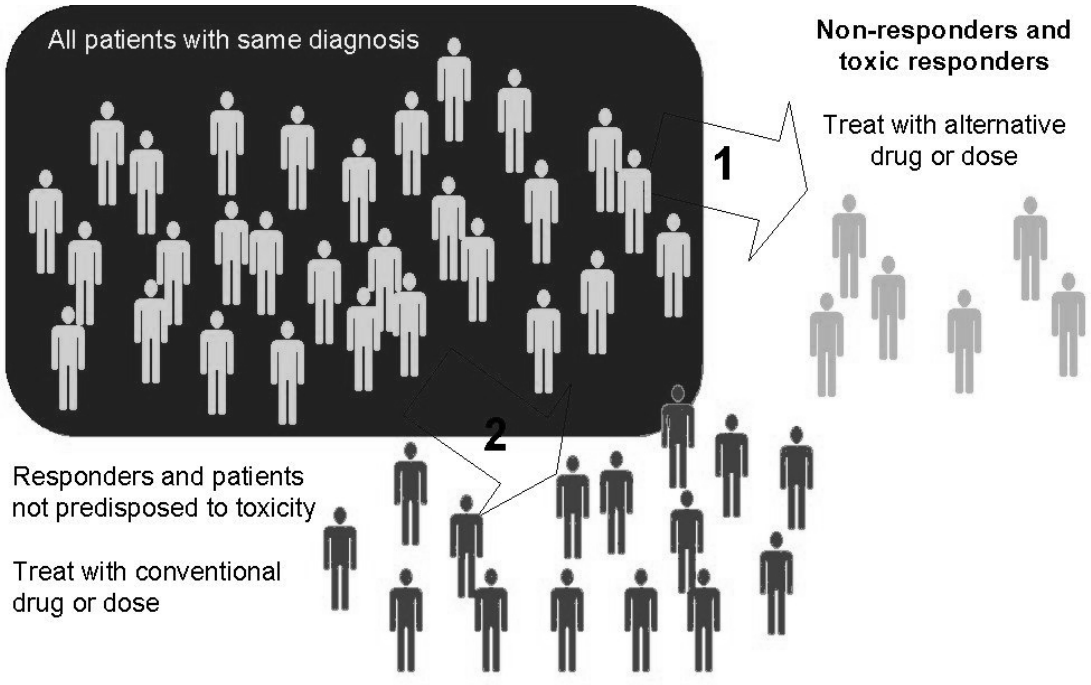

Figure 3. Potential of pharmacogenomics (Johnson, J. Pharmacogenomics.2001)

clinically relevant for predicting the unwanted or inadequate response to a particular drug as well as for predicting an increased risk. There are two approaches as to when the pharmacogenetic testing should be done.

According to one approach, the pharmacogenetic testing should only be performed when a patient shows signs of toxicity upon therapy administration or fails to respond appropriately to the therapy prescribed. According to the other approach, health care institutions should select drugs that require pharmacogenetic testing before therapy introduction. The decision is thereby based on the assessment of the polymorphic enzyme significance in the metabolism of the respective drug intended to be used in the treatment. If the drug is metabolised by a polymorphic enzyme, this being the only route of its inactivation, it can be presumed to have a major impact on the metabolism, pharmacokinetics and pharmacodynamics of the drug, and thus on its efficacy.

It seems acceptable that genotyping could be indicated in any case when the drug of choice is a substrate for a polymorphic enzyme, or in individuals exhibiting a suboptimal response to drugs that are substrates for polymorphic enzymes. The advantage of genotyping lies in the fact that it allows for the PM or UEM phenotype properties of an individual to predict, and these data can primarily prove useful for dose adjustment or choice of an alternative drug that is not the polymorphic enzyme substrate. Although the pre-dosing screening of patients would be associated with some additional cost, it would certainly be counterbalanced by reduction of the cost of treatment for toxic episodes, therapeutic failures, or subsequent interventions due to therapeutic errors

9.7 Conclusions

The advantage of pharmacogenetic testing in individual patients lies in the fact that the results provide an accurate and constant information on the patient's drug metabolizing ability before therapy introduction, thus allowing for proper dose choice and optimisation. The potential of pharmacogenomics in differentiating responders from non-responders 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 (Figure 3). The task of clinical laboratories is to adopt this new laboratory diagnostic tool that allows for detection of allele mutations and recognition of homozygous and heterozygous carriers of mutant genes.

Recommended literature:

1 Kroemer HK, Eichelbaum M. Is the gene stupid? Molecular basis and clinical consequences of genetic cytochrome P450 2D6 polymorphism. Life Sci 1995;56:2285-98.

2 Lazarou J, Pomeranz BH, Corey PN. Incidence of adverse drug reactions in hospitalized patients: a meta-analysis of prospective studies. JAMA 1998;279:1200-5.

3 Topic E. Pharmacogenetics and clinical laboratory. Biochemia Medica 1999;9:97-106.

4 Štefanovic M, Topic E, Ivaniševic A-M. Cytochrome P450 CYP2D6 genotyping by multiplex allele specific polymerase chain reaction. Biochemia Medica 1999;9:129-34.

5 Ivaniševic A-M, Štefanovic M, Topic E. PCR-SSCP method for detection of the CYP2D6 gene *3 and *4 mutations. Biochemia Medica 1999;9:123-7.

6 Meyer UA. Pharmacogenetics and adverse drug reactions. Lancet 2000;356:1667-71.

7 Meyer UA. Drugs in special patient groups: clinical importance of genomics in drug effects. In: Carruthers GS, Hoffmann BB, Melmon KL, Nierenberg DW, eds. New York: McGraw-Hill, 2000:1179-205.

8 Topic E, Štefanovic M, Ivaniševic A-M, Blazinic F, Culav J, Skocilic Ž. CYP2D6 genotyping in patients on psychoactive drug therapy. Clin Chem Lab Med 2000;38:921-7.

9 Swynghedauw B. Cardiovascular pharmacogenetics and pharmacogenomics. J Clin Basic Cardiol 2001;4:205.

10 Lee CR, Goldstein JA, Pieper JA. Cytochrome P450 2C9 polymorphisms: a comprehensive review of the in-vitro and human data. Pharmacogenetics 2002;12:251-63.

11 Chong PH, Seeger JD, Franklin C. Clinically relevant differences between the statins: implications for therapeutic selection. Am J Med 2001;111:390-400.

12 Rendic S. Human P450 metabolism database. Data searched on September 2002. http://www.gentest.com/human_p450_database/