Editorials

Pharmacogenetics and Pharmacogenomics in Cardiology

DIMITRIOS A. STAKOS, HARISIOS BOUDOULAS

Division of Cardiology, College of Medicine and Public Health, The Ohio State University, Columbus, OH, USA

Key words:
Pharmacogenetics, pharmacogenomics.

Manuscript received:
April 30, 2002;
Accepted:
June 20, 2002

Correspondence:
Harisios Boudoulas, MD, PhD

The OSU Heart and Lung Research Institute, 473 West 12th Avenue 2nd floor, 43210 Columbus, Ohio
e-mail:
Boudoulas-1@medctr.osu.edu

There is clear evidence today that efficacy and toxicity of most pharmacologic agents varies among individuals[1,2]. Differences in age, severity of underline disease, coexistent diseases, drug-drug interactions, only partially explain differences in drug response. Indeed, individuals with apparently similar characteristics may exhibit various responses to the same amount of a certain pharmacologic agent. Genetically determined factors related to drug metabolism or sensitivity of enzymes/receptors via which pharmacologic agents provide their action may play important roles [3]. Pharmacogenetics/ pharmacogenomics is the field of biomedical sciences which is studying the effect of pharmacologic agents in relation to the genetic factors[4].

Differences in drug response related to genetic factors have been reported in antiquity. Pythagoras e.g. in 510 $_{BC}$ observed that ingestion of fava beans was dangerous in certain individuals. For this reason he recommend avoidance of beans: *«...Έν τώ περί τών Πυθαγορείων παραγγέλειν αυτών απέχεσθαι τών κυάμων, ήτοι ότι Άδου πύλαις εισίν όμοιοι»*[5]. Today it is known that serious hemolytic anemia may occur in individuals with a genetically determinated deficiency of the antioxidant enzyme dehydrogenase of glucose- 6-phosphate (G-6PD) after consumption of fava beans.

Early in the 20th century, it was suggested that drugs in humans are metabolized prior to excretion.[14,15] In 1919 Marshall reported that the skin of black individuals was more resistant to mustard gas compared to the skin of white individuals. During World War II, it was observed that administration of antimalarial drugs produced hemolytic anemia more often in African-Americans compared to Caucasians[16]. In 1956, it was demonstrated that G6PD deficiency is the major factor related to hemolytic anemia after administration of antimalarial drugs[17]. G6PD deficiency occurs more often in African compared to white Americans and it is genetically determined. In 1968 it was shown that plasma half-lives of many drugs are remarkably similar in monozygotic twins, whereas a wide variation in drug half-lives were observed in dizygotic twins, siblings and in the general population[18].

In this review, the principles of pharmacogenetics /pharmacogenomics and their applications to cardiology will be discussed briefly. To better understand pharmacogenetics a few principles related to genetics and single nucleotide polymorphism will be presented.

Genetics

Hippocrates was the first to describe that characteristics in children are related to both of their parents. *«...Έστι δέ ούκ ανυ-*

στόν πάντα τη μητρί εοικέναι τώ δέ πατρί μηδέν, ή τό εναντίον τούτου, ουδέ μηδετέρω εοικέναι μηδέν· αλλά αμφοτέροισιν ανάγκη τίς εστίν εοικέναι τινί»[6]. He also mentioned that children may have the same disease with their parents. *«... Άρχεται δέ ώσπερ καί τάλλα νουσήματα κατά γένος. Τί κωλύει ότω πατήρ καί μήτηρ είχετο, τούτο τό νοσήματι καί τών εκγόνων έχεσθαι τινά;»*[7].

Mendel was the first to have described the laws of inheritance in 1866[9]. Modern genetics, however, started in 1953 when James D Watson and Francis HC Crick defined the structure of deoxy-rivose nucleic acids (DNA)[10]. DNA is composed of two polynucleotide chains that form a double helix around the same central axis and run in opposite directions. Each chain consists of phosphate diester groups joining deoxyribose residues and nitrogen bases. The bases are on the inside of the helix and the phosphates on the outside. The planes of the bases are perpendicular to the central axis. The bases are joined together in pairs, a single base from each chain. One of the pair must be a purine and the other pyrimidine for a bonding to occur (Figure 1). Only specific pairs of bases can bond together. These pairs are adenine (purine) with thymine (pyrimidine), and guanine (purine) with cytosine (pyrimidine). The sequence of bases on a single chain does not appear to be restricted in any way. However, if only specific pairs of bases can be formed, it follows that if the sequence of bases on one chain is known, then the sequence on the other chain is automatically determinated[11].

The specific DNA sequence that encodes a protein is called gene. Genes are the biological unit of inheritance. The location of a gene on a chromosome is called locus (from the Latin word which means "place"). A specific triplet of bases in the DNA chain is translated to an amino acid and is called a codon. The correspondence between specific codons and amino acids is known as the genetic code. Two genes located near one another on the same chromosome tend to be transmitted together rather than independently. These genes are said to be linked (Figure 2).

While DNA is located in the cell nucleus, protein synthesis takes place in the cytoplasm. The information contained in DNA must thus be transferred to the cytoplasm. This involves a process called transcription. During transcription, the DNA code is transcribed into messenger RNA (mRNA), which leaves the nucleus to be translated into proteins. Initially the transcription generates the primary mRNA. Excision of the introns and subsequent

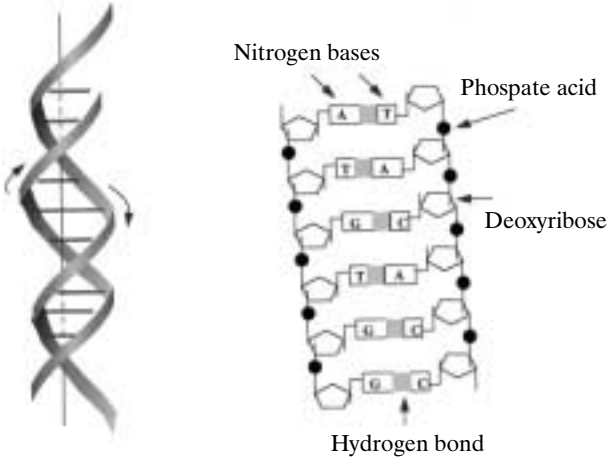

Figure 1. Structure of deoxyribose nucleic acid (DNA). **Left:** Schematic presentation of the double helix of DNA. The two strands run in opposite directions (arrows). The nitrogen bases and the hydrogen bonds are presented schematically as perpendicular lines, to the central axis. The whole structure resembles a ladder rotated to its longitudinal axis. **Right:** The chemical structure of DNA. The backbone of DNA consists of regularly repeated deoxyribose sugars and phosphates. The deoxyribose sugars are joining together by phosphodiester bonds. A nitrogen base is stacked to each sugar in a plane perpendicular to the helical axis. Hydrogen bonds between bases of opposite strands are holding the two strands together. A=adenine; T=thymine; G=guanin; C=cytocine (see text for details).

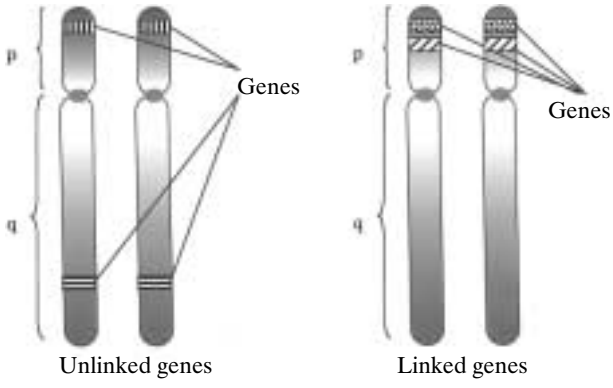

Figure 2. Schematic presentation of homologous chromosomes with two pairs of genes located in corresponding loci. These genes are called alleles. The p and q indicate the short and long arm of each chromosome respectively. **Left:** There is a pair of alleles on each arm of the chromosome. The two genes are located far apart On the same chromosome, in distant loci (unlinked genes). **Right:** the two genes are located in adjacent loci on the same chromosome (linked genes). Due to proximity between them, the likelihood of passing together to the next generation after genetic recombination is high.

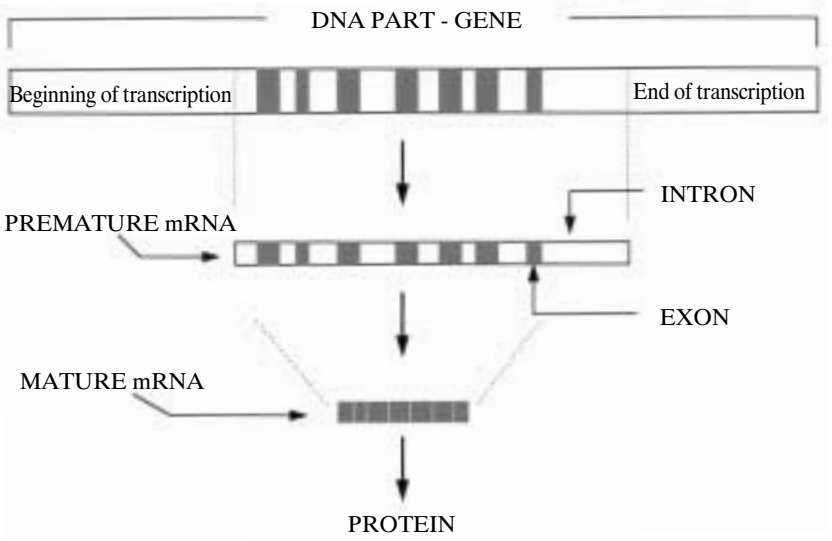

Figure 3. The first step for the DNA transcription to messenger ribose nucleic acid (mRNA) is the formation of a premature mRNA. Special enzymes cut out noncoding sequences called introns. The remaining sequences form the mature mRNA and are called exons.

joining of the exons results in the formation of the mature mRNA (Figure 3). The mature mRNA is then transported out of the nucleus for translation.

The total number of human genes composes the human genome[8]. Genes among the same species are almost identical. Mutation is defined as a transmittable change in DNA sequence. Single gene mutations represent substitution, deletion or insertion of a base in a gene. This can result in a change in amino acid sequence and a structural or functional defect in the encoded protein.

A single gene mutation can be determined by three elements: the bases that have been changed and the position of the mutation on the gene. In angiotensinogen gene for example, T704-C or T704 ˚C denotes that in the position 704 thymine (T) is changed to cytosine (C). This mutation results in the substitution of methionine (Met) to threonine (Thr) in the angiotensinogen molecule. The same mutation can then be determined as Met235-Thr or Met235 ˚Thr.

Several genetic diseases like the Marfan syndrome, cystic fibrosis, neurofibromatosis, muscular dystrophy etc, are the result of a mutation in a single gene. The frequency of these mutations is limited (<1%) because the survival of these individuals is reduced due to the disease process.

Often, however, changes in DNA sequence do not lead directly to a serious disease. Those genetic variations are found frequently in the general population. Inherited variations that occur at a frequency greater than 1% are called polymorphisms. Polymorphisms may contribute to traits such as skin or eye color, red blood cells antigens, but may also contribute to susceptibility of certain diseases and/or to differences in response to pharmacologic agents.

Polymorphisms that involve change in one in nitrogen base are called single nucleotide polymorphisms (SNPs). It is estimated that SNPs occur in one in every 1000 bases[12,13]. The total number of bases in the human genome is approximately 3×10^9. Thus the total number of SNPs is estimated to be approximately 3 million.

Certain polymorphisms may be of a great clinical significance. For example in glycoprotein (GP) IIb/ IIIa platelet receptor polymorphism there is a cytosine for thymine substitution in position 1565 of the gene. This change causes a leukine to proline substitution in position 33 of the polypeptidic chain. Platelets carrying the polymorphic IIb/IIIa receptors demonstrate increase aggregation compared to platelets carrying the wild type (without mutation) receptors. The effect of some drugs like aspirin and estrogen on platelet aggregation may also be different in relation to IIb/ IIIa receptor polymorphism (Figure 4).

Changes in bases within the coding regions of a gene may alter the structure and function of the encoded protein. Changes, however, may occur without any implication on the structure or function of the encoded protein. These mutations are called silent mutations and usually occur in the non-coding regions of a gene. In some cases (angiotensin II receptor polymorphism), even silent mutations may be related to susceptibility of specific diseases or have effect on drug action. In these cases, the gene with

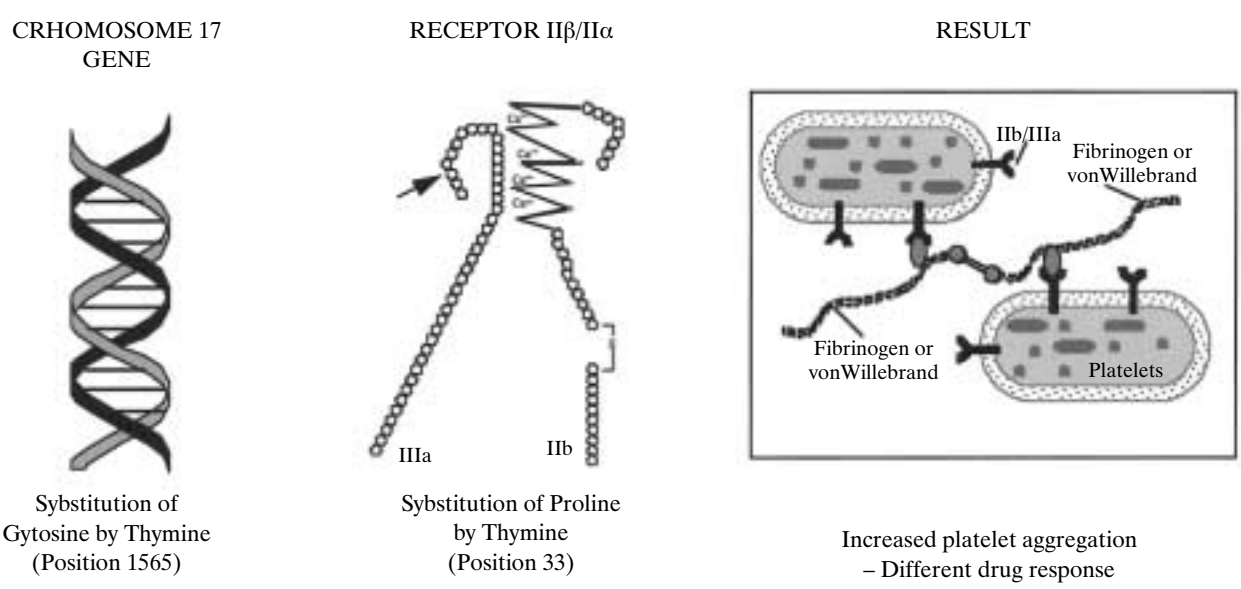

Figure 4. Left: Gene of glycoprotein (GP) platelet receptor IIb/IIIa in chromosome 17 (schematic presentation). A substitution of cytosine by thymine in position 1565 of the gene encoding IIb/IIIa receptor, results in two polymorphic proteins. **Centre:** Schematic presentation of GP IIb/IIIa receptor molecule. There is a leukine to proline substitution in position 33 of the polypeptidic chain. **Right:** This aminoacid substitution causes increased platelet aggregation and different drug response.

the silent mutation is linked with another gene, which is responsible for the phenotypic expression.

Pharmacogenetics

Genetic factors may affect drug response by two major mechanisms: a) alteration of drug metabolism; b) alteration of the sensitivity of enzymes or receptors by which pharmacologic agents provide their effects.

Drug Metabolism

Drugs are most often eliminated by biotransformation and/or excretion into the urine or bile. The metabolic transformation of drugs is catalyzed by enzymes, mainly in the liver. Many drugs used in cardiology are metabolized by two enzymes: N-acetyl-transferase and cytochrome P-450 (Table 1).

N acetyltransferase (NAT). NAT-1 and NAT-2 are involve in the metabolism of several drugs by transferring one acetyl group from acetyl coenzyme A to target amines with a final result in the production of an amide. This process increases the hydrophilicity of the drugs and enhances its elimination by the kidneys. The gene of NAT-2 is located in chromosome 8[19] (Table 2). An adenine to thymine substitution in position 590 of NAT-2 gene results in an arginine to glutamine substitution in position 197 of the polypeptidic chain of the enzyme which causes a decrease in the activity of the enzyme[20]. Many polymorphisms of NAT-2 gene have been described[21]. Populations can be divided into rapid or slow acetylators. The frequency of rapid acetylators is different in different populations[22-24].

Procainamide is another drug which is metabolized by NAT-2. N-acetylprocainamide (NAPA) is the product of metabolism; it has antiarrythmic activity and is excreted by the kidneys[25,26]. Thus, in fast acetylators high NAPA levels and adverse effects related to NAPA may occur, especially in patients with renal insufficiency. In slow acetylators with decreased enzyme activity, plasma concentrations of drugs metabolized by NAT are higher compared to fast acetylators. Antinuclear antibodies and lupus-like syndrome occur more often in slow compared to fast acetylators during therapy with hydralazine or procainamide[25].

Cytochrome P-450 (CYP-450). More than 50 commonly used cardiovascular drugs are metabolized by CYP-450 enzyme family (Table 1).

Polymorphisms of the CYP-450 have important clinical implications. CYP-450 2C9 is the principal enzyme that catalyzes the conversion of the pharmacologically more potent S-enantiomer of warfarin to its inactive metabolites. The less potent R-enan-

Table 1. Pharmacologic Agents Metobolized via N-acetyltransterase (NAT). Cytochrome (CYP) 450.
Cardiovascular drug metabolism in N-acetyltransferase (NAT) and cytochrome P450 (CYP450) enzymes. Many cytochrome P450 gene families (isoenzymes) have been identified in humans (CYP450-1, 2, 3, etc). The sequence of aminoacids between enzymes of the same family is at least 40% identical. The families are further divided in subfamilies (CYP450-1A2, 2A6, 2C9, 2C19, etc). Isoenzymes of the same subfamily have >55% identical aminoacid sequences. The cytochrome P450-1, 2 and 3 families are involved in the biotransformations of the majority of drugs, whereas the rest of the families are important for the metabolism of endogenous substances like steroids, fatty acids and others.

NAT	CYP-450 1A2	CYP-450 2A6	CYP-450 2C9	CYP-450 2C19	CYP-450 2D6	CYP-450 3A4
Procainamide	Warfarin-R	Coumarin	Warfarin-S	Propranolol	Propranolol	Amiodarone
Hydralazine	Caffeine	Nicotine	Irbesartan		Metoprolol	Digitoxin
	Mexiletine		Losartan		Carvedilol	Lidocain
	Verapamil		Fluvastatin		Timolol	Losartan
					Captopril	Lovastatin
					Encainide	Quinidine
					Flecainide	Verapamil
					Mexiletine	Diltiazem
					Propafenone	Nifedipine
						Amlodipine
						Felodipine
						Nisoldipine
						Nitredipine

tiomer is metabolized by CYP-450 1A2 and 1A3[27]. CYP-450 2C9 gene is located in chromosome 22. An adenine to thymine substitution in position 3023 of the gene causes a histidine to proline substitution in position 144 of the polypeptidic chain of the enzyme (Table 2). This genetic polymorphism produces variants with different enzymatic activity. Individuals with the polymorphism require a very low dose of warfarin, and have an increased risk of bleeding complications compared to individuals carrying the wild type enzyme[28]. Knowledge of CYP-450 2C9 genotype may help to better manage patients treated with warfarin.

Nicotine is the primary compound of tobacco responsible for its dependence. In humans, 60-80% of nicotine is metabolized to cotinine mostly by the CYP2A6 enzyme. The gene coding for *CYP2A6* is located in chromosome 19. A thymine to adenine substitution in position 488 of the gene causes a leukine to histidine substitution in position 160 of the polypeptidic chain of the enzyme. The result of this polymorphin is the production of an inactive enzyme. Individuals lacking full function of CYP2A6, have impaired nicotine metabolism and are protected for becoming tobacco-dependent. In addition, smokers whose nicotine metabolism is impaired, smoke fewer cigarettes than those with normal nicotine metabolism[29]. Smoke produced from cigarettes contains nitrosamines which are carcinogens; individuals who carry CYP2A6 inactive enzyme are less likely to develop lung cancer[30].

Table 2. Polymorphisms Affecting Metabolism of Cardiovascular Drugs.

Enzyme	locus	Base	Aminoacid	Polymorphism frequency	Polymorphism result
N-acetyl-transferase	8p22	Guanine instead of Adenine position 590	Gloutamine instead of Arginine position 197	Wild type ~ 70% Heterozygotes ~ 30% Homozygotes ~ 0%	Decreased enzyme activity
Cytochrome P4502C9	22q12	Cytocine instead of Adenine position 3023	Histidine instead of Proline position 144	Wild type ~ 60% Heterozygotes ~ 40% Homozygotes ~ 0%	Decreased enzyme activity
Cytochrome P4502A6	19	Adenine instead of Thymine position 488	Histidine instead of Leukine position 160	Wild type ~ 87% Heterozygotes ~ 12.5% Homozygotes ~ 0.5%	Decreased enzyme activity

Enzymes and/or Receptors Related to Pharmacologic Action of Drugs (Table 3)

Renine - Angiotensin - System (RAS)

Renin is a protease which is synthesized and stored in the kidneys. Renin converts angiotensinogen to angiotensin I. Angiotensin Coverting Enzyme (ACE) converts angiotensin I to angiotensin II. Angiotensin II has vasoconstrictive effects and stimulates the release of aldosterone from the adrenal cortex. Angiotensin II provides its pharmacological effect via specific cell surface receptors AT1 and AT2. Polymorphisms in genes coding for angiotensinogen, ACE, or angiotensin II receptors have been described (Figure 5)[31].

Angiotensinogen. Human angiotensinogen contains 452 amino acids. The gene encoding angiotensinogen is located in chromosome 1. A thymine to cytosine substitution in position 704 of the gene causes the substitution of methionine for threonin in position 235 in angiotensinogen molecule (Table 3)[32]. Thus, there are three possible genotypes: methionine-methionine (MM, wild type), methionine-threonine (MT, mutant form, heterozygotes) and threonine-threonine (TT, mutant form, homozygotes)[33]. Individuals with the TT genotype have increased angotensinogen and angiotensin II plasma levels[34]. For this reason hypertensive patients carrying the polymorphic form of angiotensinogen require higher doses of ACE inhibitors for similar therapeutic results, compared to patients carrying the wild type of angiotensinogen[35,36].

The reduction of renal blood flow during angiotensin II infusion in renal arteries is greater in individuals carrying the wild type angiotensinogen compared to those carrying the angiotensinogen with polymorphism. This phenomenon is probably related to a decrease of the number of angiotensin II receptors in renal arteries (down regulation) due to chronic stimulation from angiotensin II[37,38].

Angiotensin Converting Enzyme (ACE). The gene encoding ACE is located in chromosome 15 (Table 3)[39]. The ACE gene polymorphisms characterized by either insertion (I) or deletion (D) of a 287-base pair sequence in intron 16. The three possible genotypes are: II, ID and DD (Figure 6). Individuals with the DD genotype have increased plasma ACE levels, while individuals with II genotype have lower ACE plasma levels[40,41]. The DD genotype has been associated with increased risk of myocardial infarction[42,43], greater left ventricular dilatation after anterior myocardial infarction[44], greater left ventricular hypertrophy in hypertensive patients[45] and reduced survival in patients with heart failure[46].

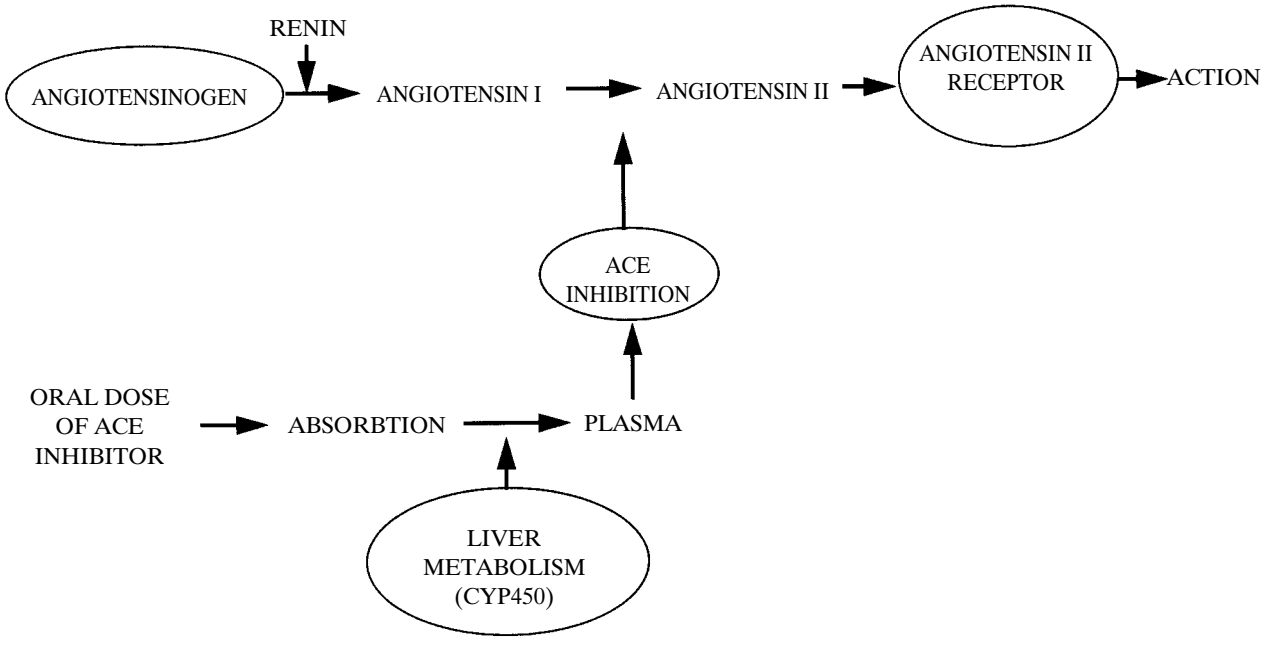

Figure 5. Renin acts on angiotensinogen to catalyze the formation of angiotensin I. Angiotensin II converting enzyme (ACE) converts angiotensin I to angiotensin II. Angiotensin II acts via the angiotensin II receptors. Following an oral dose of ACE inhibitor, the drug is absorbed and metabolized in the liver.

Table 3. Polymorphism Altering Effect of Cardiovascular Drugs.

Protein	locus	Base	Aminoacid	Polymorphism frequency	Polymorphism result
Angiotensinogen	1q43	Cytosine instead of Thymine position 704	Threonine instead of Methionine position 235	Wild type ~ 30% Heterozygotes ~ 52% Homozygotes ~ 18%	Increased angiotensinogen levels
Angiotensinogen	1q43	Guanine instead of Adenine position 6	Silent mutation	Wild type ~ 30% Heterozygotes ~ 52% Homozygotes ~ 18%	Increased angiotensinogen levels
Angiotensin Converting Enzyme (ACE)	17q23	287 base pair insertion/deletion intron 16	Silent mutation	Insertion-Insertion (II) ~ 20% Insertion-Deletion (ID) ~ 45% Deletion-Deletion (DD) ~ 35%	Increased ACE plasma levels
Angiotensin receptor T_1	3q21	Cytosine instead of Adenin position 1166	Silent mutation	Wild type ~ 50% Heterozygotes ~ 41% Homozygotes ~ 9%	Increased sensitivity to angiotensin
Bradykinine receptor	14q32	Cytosine instead of Thymine position 58	Silent mutation	Wild type ~ 15 % Heterozygotes ~ 60% Homozygotes ~ 25%	Decreased receptor synthesis
Nitric oxide (NO) synthase	7q35	Thymine instead of Guanine position 894	Aspartic acid instead of Gloutamine position 298	Wild type ~ 40% Heterozygotes ~ 43.5% Homozygotes ~ 16.5%	Decreased enzyme activity
Alpha adducin	4p16	Thymine instead of Guanine position 614	Tryptophane instead of Glycine position 460	Wild type ~ 60% Heterozygotes ~ 25-35% Homozygotes ~ 4%	Increased sodium re-absorption
Epithelial sodium channels	16p12	Thymine instead of Cytocine position 1781	Methionine instead of Threonine position 594	Heterozygotes: Whites ~ 0.6% Africans ~ 3% Homozygotes ~ 0%	Increased sodium re-absorption
β_1-adrenergic receptor	10q24	Guanine instead of Adenine position 145	Glycine instead of Serine position 49	Wild type ~ 65% Heterozygotes ~ 25% Homozygotes ~ 10%	Decreased receptor sensitivity
β_1-adrenergic receptor	10q24	Guanine instead of Cytosine position 1165	Arginine instead of Glycine position 389	Arginine 389 ~ 74% Glycine 389 ~ 26%	Increased receptor sensitivity
β_2- adrenergic receptor	5q31	Thymine instead of Cytosine position 491	Isoleukine instead of Threonine position 164	Wild type ~ 97% Heterozygotes ~ 3% Homozygots ~ 0%	Decreased receptor sensitivity
G_s protein	20	Cytosine instead of Thymine codon 131	Silent mutation	Wild type ~ 27% Heterozygotes ~ 55% Homozygotes ~ 18%	Decreased coupling with adrenergic receptors
Platelet receptor IIb/IIIa	17q21	Thymine instead of Cytosine position 1565	Proline instead of Leukine position 33	Wild type ~ 73% Heterozygotes ~ 25% Homozygotes ~ 2%	Increased platelet aggregation
Platelet receptor FcRIIa	17q21	Adenine instead of Guanine position 507	Histidine instead of Arginine position 131	Homozygotes (Histidine) ~ 28% Heterozygotes ~ 52% Homozygotes (Arginine) ~ 20%	Increased receptor activity
Cholesteryl-ester-tranferase (CETP)	16q21	Adenine instead of Guanine position 279	Silent mutation	Wild type ~ 35% Heterozygotes ~ 50% Homozygotes ~ 15%	Decreased enzyme activity
stromelysin –1	11q2	Insertion of Adenine position 1171	Silent mutation	Wild type ~ 25% Heterozygotes ~ 50% Homozygotes ~ 25%	Increased enzyme activity

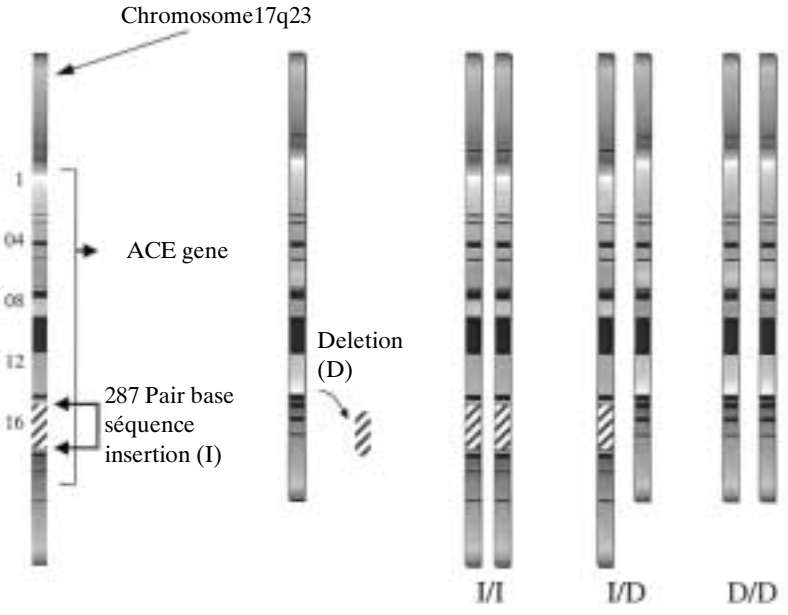

Figure 6. Left: Angiotensin converting enzyme (ACE) gene locus at chromosome 17 (schematic presentation). The introns of the gene are marked with numbers 1 to 16. A 287 pair base sequence is located in intron 16. The presence or absence of this sequence determing the insertion (I) or deletion (D) polymorphism respectively. **Right:** The three possible genotypes: II, ID and DD.

The response to ACE inhibitors is also affected by ACE genotype. Therapy with captopril in post-myocardial infarction patients blunted left ventricular dilatation in DD genotype patients but not in ID or II[44]. Similarly, regression of left ventricular hypertrophy with enalapril in hypertensive patients was greater in those carrying the DD compared to the other two genotypes[47-49].

Angiotensin II receptor. The gene encoding angiotensin II receptor is located in chromosome 3 (Table 3). A silent polymorphism (adenine to cytosine substitution in position 1166) has been described[50]. The homozygous form of this polymorphism (cytosine-cytosine, CC) is associated with decreased aortic elasticity compared to other two genotypes (AA and AC)[51,52]. Treatment with ACE inhibitors improves aortic elastic properties in CC and AC carriers but not in AA carriers. In contrast, calcium channel blockers improve elastic properties of the aorta in AA but not in AC or CC genotypes[53]. Further, treatment with losartan, an angiotensin II receptor blocker was associated with significantly greater decrease of mean arterial pressure in AC and CC individuals compared with AA individuals[54].

B_2 bradykinin receptor. The most common adverse effect during therapy with ACE inhibitors is a dry, persistent cough. The cause of this adverse effect has been attributed to a local accumulation of bradykinin[55]. Speculations about a genetic determination of this adverse effect have implicated polymorphisms of the genes encoding ACE (higher prevalence of cough in II genotype) and bradykinin B_2 receptor[56].

The gene encoding for bradykinin B_2 receptor is located in chromosome 14 (Table 3). A thymine to cytosine substitution has been described in position 58 of the gene[57]. The substitution is located in the promoter region (non-coding region, silent polymorphism). However the promoter region is responsible for the transcriptional rate of the gene. Individuals carrying cytosine in position 58 have less bradykinin receptors, decreased sensitivity to bradykinin, and possibly lower frequency of ACE inhibitors induced cough[58].

Nitric Oxide

In endothelial cells, the endothelial derived relaxing factor, nitric oxide (NO), is formed from L-arginine by endothelial NO synthase (eNOS). Inhibition of eNOS and impairment of NO production may facilitate the atherogenic process[59]. The gene encoding eNOS is located in chromosome 7 (Table 3)[60]. A guanine to thymine substitution in position 894 of the gene causes a glutamine to aspartic acid substitution in position 298 of the polypeptidic chain of eNOS. The mutant form of the eNOS has decreased enzyme activity. This polymorphism has been associated with increased risk for carotid atherosclerosis[61], myocardial infarction[62] and arterial hypertension[63,64]. In addition, the aspartic acid-298 polymorphism may predispose resistance to conventional antihypertensive therapy in patients with arterial hypertension[65].

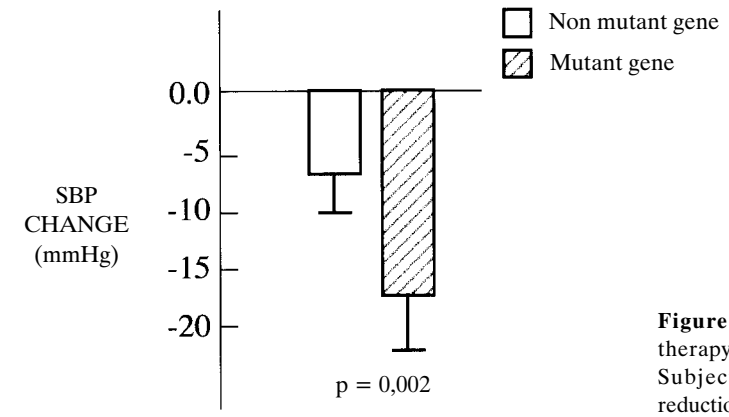

Figure 7. Changes in systolic blood pressure (SBP) during therapy with thiazides, according to α-adducin polymorphism. Subjects carrying the mutant gene shown a greater SBP reduction.

Sodium channels

Sodium and extracellular-fluid homeostasis are maintained by regulation of sodium reabsorption by the kidneys. Sodium channels are responsible for sodium reabsorption along the renal tubules. Alpha-adducin and epithelial sodium channels are important for sodium reabsorption.

Alpha-adducin. Adducin regulates the function of sodium-potassium pump. The gene encoding alpha adducin is located in chromosome 4 (Table 3)[66]. A guanine to thymine substitution in position 614 of the gene, causes a glycine to tryptophane substitution in position 460 of the polypeptidic chain of adducing molecule. The presence of this polymorphism is associated with increase sodium reabsorption and arterial hypertension. Moreover, hypertensive patients carrying the tryptophane-460 polymorphism have a better response to therapy with diuretics compared to those with the wild type adducin (Figure 7)[67,68].

Epithelial sodium channels. The amiloride-sensitive epithelial sodium channels are located in the distal renal tubules. Epithelial sodium channels are basically formed from three subunits (alpha, beta and gamma). The gene encoding the beta subunit is located in chromosome 16 (Table 3). A cytosine to thymine substitution in position 1781 of the gene causes a threonine to methionine substitution in position 594 of the beta subunit molecule[69]. This polymorphism increases sodium channel activity, renal tubular sodium reabsorption and may result in the development of arterial hypertension. Methionine-594 beta subunit polymorphism occurs more frequently in black individuals with arterial hypertension compared to those without hypertension[70]. Identification of this polymorphism in hypertensive individuals may allow a more rational approach to the treatment with drugs such as amiloride, a specific blocker of these channels.

Adrenergic receptors

Catecholamines endogenous or exogenous provide their affects via adrenergic receptors.

β1 Adrenergic Receptors. The gene encoding for β1 adrenergic receptors is located on chromosome 10 (Table 3)[71]. An adenine to guanine substitution in position 145 of the gene causes a glycine to serine substitution in position 49 of the receptor molecule. The serine-49 polymorphism is associated with decrease mortality risk in patients with congestive heart failure[72]. Treatment with β-blockers was associated with a significantly greater increase in survival in patients carrying the serine-49 polymorphism than in those carrying the wild type receptor (Figure 8).

A second polymorphism of β1 adrenergic receptors is caused by a cytosine to guanine substitution in position 1165 of the gene. This is associated with a glycine to arginine substitution in position 389 of the receptor molecule. In vitro studies have showed a twofold greater basal and threefold greater agonist mediated activity with the arginine-389 receptor form. Moreover, a recent study showed that mean resting heart rate, resting systolic and diastolic blood pressure and double product (heart rate x systolic blood pressure) were significantly greater in arginine-389 (homozygous) individuals compared to

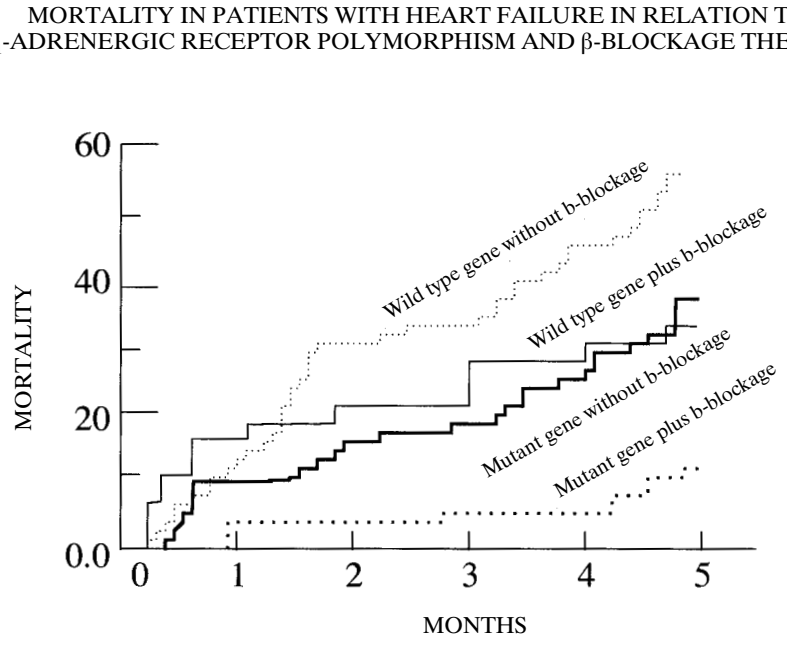

Figure 8. Mortality in patients with heart failure in relation to β_1-adrenergic receptor polymorphism and β-blockade therapy. Patients with the mutant gene are represented by (+), and those with the wild type gene are shown with (–). Patients receiving β-blockade therapy are represented by (+), and those without β-blockade therapy are shown with (–). Patients with the mutant gene receiving β-blockade therapy (+) (+) have the best prognosis, while patients with the wild type gene without β-blockade therapy (–) (–) have the worst prognosis. Patients with the mutant gene not receiving β-blockade therapy (+) (–) have similar mortality compared to patients with the wild type gene who receive β-blockade therapy (–) (+). β-Blockade therapy is more effective in patients with β_1 receptor polymorphism.

glycine carriers (homozygotes and heterozygotes)[73]. There is also evidence that individuals with the variant exhibit a greater increase in blood pressure and heart rate during dobutamine stimulation.

β2 Adrenergic Receptors. The gene encoding β2 adrenergic receptors is located on chromosome 5 (Table 3). A cytosine to thymine substitution in position 491 of the gene causes a threonine to isoleukine substitution in position 164 of the β2 adrenergic receptor molecule. This polymorphism is associated with decreased activity of the receptor due to defective coupling of the receptor to the stimulatory G protein. The isoleukine-164 variant is associated with decreased survival in patients with congestive heart failure[74].

G Proteins. Adrenergic as well as other plasma membrane receptors act by binding guanosin triphosphate (GTP) to specific G proteins, which are located on the inner face of the plasma membrane. GTP binding activates the G protein, such that it in turn can regulate the activity of adenylyl cyclase. G proteins stimulate (G_S) or inhibit (G_I) adenylyl cyclase. Adenylyl cyclase is responsible for cyclic adenosin monophosphate (cAMP) production. cAMP is a protein messenger present in many different types of cells, which activates cAMP-dependent protein kinases, causing them to transfer phosphate groups from free molecules of ATP to various proteins in the cell. G proteins are consistent from three subunits: alpha, beta and gamma. The gene encoding for the alpha subunit of Gs protein is located on chromosome 20 (Table 3). A silent polymorphism has been described from a thymine to cytosine substitution in codon 131. Hypertensive patients carrying this polymorphism exhibit a greater reduction in blood pressure with β-blockade therapy compared to patients with the wild type Gs protein[75].

Platelet receptors

Rupture of an atherosclerotic plaque leads to the formation of a platelet-rich thrombus and subsequently to acute ischemic syndrome. Platelet aggregation is mediated through the binding of fibrinogen and von Willebrand factor to the activated form of the platelet glycoprotein (GP) IIb/IIIa receptors. The gene encoding the IIIa subunit of this receptor is located on chromosome 17 in adjacent locus to ACE gene (Table 3)[76]. A cytosine to thymine substitution in position 1565 of the IIIa gene causes a leukine to proline substitution in position 33 of the polypeptidic chain of the receptor ($PI^{A1/A2}$ polymorphism)[77]. PI^{A2} polymorphism (proline-33) is associated with increased platelet aggregation and increased risk of ischemic events[78].

Aspirin. Aspirin is widely used for the prevention and treatment of acute coronary syndromes and cerebrovascular accidents[79,80]. The inhibition of platelet aggregation by aspirin depends on the $PI^{A1/A2}$ polymorphism. PI^{A2} platelets display a lower threshold

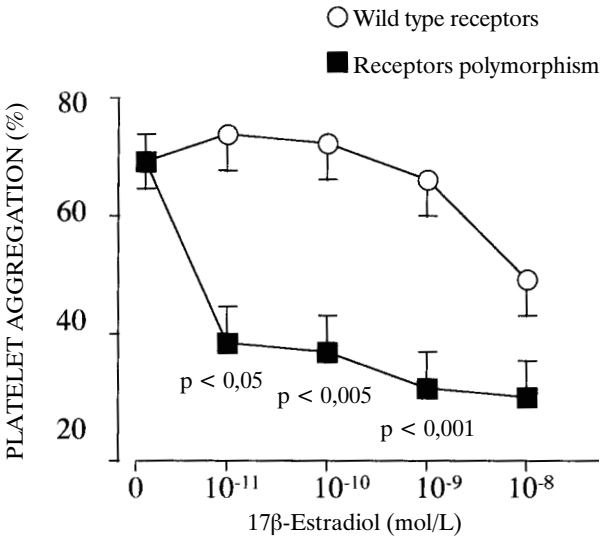

Figure 9. Effect of 17β-estradiol on platelet aggregation in relation to glycoprotein (GP) IIb/IIIa receptor polymorphism (PlA1/A2). Platelets with the wild type receptors (A1A1) demonstrate a lower inhibition with 17β-estradiol compared to A1A2 platelets.

for activation and $PI^{A1/A2}$ heterozygous platelets shown increased sensitivity for inhibition to aspirin. PI^{A1} platelets are resistant to aspirin[81,82]. Patients with coronary heart disease carrying PI^{A1A1} platelets may require an alternative to aspirin antiplatelet therapy, such as clopidogrel.

Estrogen. The progression of ischemic heart disease accelerates markedly after menopause[86]. This is partially due to a drop of endogenous estrogen production by the ovaries[87,88]. Administration of estrogen in post-menopausal women, however, with coronary heart disease was associated with a slight increase in the frequency of coronary events during the first year after the initiation of therapy[89]. Studies in Heart and Lung Institute at the Ohio State University have shown that estrogen inhibits platelet aggregation, but such inhibition is genetically determined. $PI^{A1/A2}$ platelets shown an increased response (inhibition of aggregation) to estrogen compared to $PI^{A1/A1}$ platelets (Figure 9)[90]. In addition, $PI^{A1/A1}$ platelets have shown slightly increased platelet aggregation with small concentrations of estrogen.

Statins. The presence of $PI^{A1/A2}$ polymorphism is associated with increased risk of restenosis and ischemic events after coronary angioplasty compared to $PI^{A1/A1}$ polymorphism[83]. Therapy with statins is more effective in reducing restenosis in patients with $PI^{A1/A2}$ compared to patients with $PI^{A1/A1}$ platelets[84].

Heparin

Heparin-induced thrombocytopenia is a severe complication and is due to the development of IgG antibodies that recognize a complex of heparin and platelet proteins, in most cases platelet factor 4. These antibodies activate platelets via the platelet FcγRIIa receptors. FcγRIIa receptor polymorphisms are related to the frequency and severity of heparin-induced thrombocytopenia. The gene encoding FcγRIIa receptor is located on chromosome 1 (Table 3). A guanine to adenine substitution in position 507 of the gene causes an arginine to histidine substitution in position 131 of the receptor molecule. The histidine-131 polymorphism is associated with decreased clearance of immune complexes, prolonged activation of platelets and endothelial cells and increased risk of severe heparin-induced thrombocytopenia with thrombotic complications[85].

Cholesteryl-ester transfer protein (CETP)

CETP plays a key role in the metabolism of high-density lipoprotein (HDL) cholesterol. High plasma concentrations of CETP are associated with reduced concentrations of HDL cholesterol[91]. The gene encoding CETP is located to chromosome 16 (Table 3). There is a silent polymorphism due to a guanine to adenine substitution in position 279 of CETP gene[92].

Individuals carrying the adenine-279 polymorphism have lower HDL levels and an increased risk of coronary atherosclerosis, compared to those with guanine-279[93]. In addition, this polymorphism appears to predict whether men with coronary heart disease will benefit from treatment with pravastatin to delay the progression of coronary atherosclerosis. Individuals carrying the adenine-279 polymorphism have a greater benefit from statins compared to those with adenine-279[94].

Matrix metalloproteinases (MMPs)

Although the mechanisms underlying plaque rupture are unclear, MMPs may contribute to the weakening of the fibrous cap, which predispose to plaque rupture. MMPs comprise a number of proteases such as collagenase, gelatinase and stromelysin[95]. The gene encoding stromelysin-1 is located on chromosome 11 (Table 3). Recently, a silent po-

lymorphism has been described in which there is a sequence of six adenosine bases instead of five (there is an insertion of an adenine base in position 1171-promoter region)[96]. This polymorphism is associated with an increased prevalence of myocardial infarction and increased restenosis rate after coronary angioplasty[97,98]. Statin therapy reduces the restenosis rate significantly in patients carrying the polymorphism compared to those with the wild type of stromelysin-1[99].

Pharmacogenomics

Pharmacogenomics represent a natural evolution of pharmacogenetics. Unlike pharmacogenetics, which study the genetic variation of one gene in relationship to drug function, pharmacogenomics are generally used for more sophisticate approaches and relate the entire genotype (genome) to the drug response[98-101].

Drug metabolism may be related to genetic factors. The same dose of a drug in a slow metabolizer will give high plasma concentrations, but low plasma concentrations in a fast metabolizer. In addition to metabolism, different sensitivity of enzymes or receptors by which pharmacologic agents provide their action may alter drug effect. Individuals carrying polymorphisms associated with increased metabolism rates and decreased receptor sensitivity will require higher doses to achieve a desired pharmacological effect. In contrast, individuals carrying polymorphisms associated with decreased drug metabolism and increased receptor sensitivity will require lower doses of a certain drug to achieve a therapeutic effect.

It is known that many polygenic diseases, such as arterial hypertension, atherosclerosis and diabetes mellitus may be associated with a variety of genetic differences. These differences in genetic material may also alter the effect of pharmacologic agents. For example, in patients with arterial hypertension and increased activity of the renin-angiotensin system (ACE, angiotensinogen or angiotensin II receptor polymorphisms) therapy with ACE inhibitors may be more beneficial compared to other antihypertensive agents. In contrast, in hypertensive patients with increased sympathetic nervous system activity, therapy wtih b-blocking agents may be more appropriate, while in patients with increased sodium reabsorption (alpha-adducin or sodium channel polymorphisms), therapy with diuretics may be more effective.

Multiple combinations of these polymorphisms may exist. Thus, an individual may demonstrate rapid metabolism of anti-hypertensive drugs, high activity of renin-angiotensin system, high sympathetic nervous system activity and increased sodium reabsorption. These individuals are at high risk for developing arterial hypertension. In contrast, individuals with low renin-angiotensin activity, low sympathetic nervous system activity and normal or low sodium reabsorption are at low risk for developing arterial hypertension. Response to therapy in these two different types of arterial hypertension will also be totally different. Pharmacogenomics is also expected to help in the development of new drugs. Genetic maps, expression arrays (proteomics) and molecular methods could be used to structure highly specified therapeutic agents without adverse effects[102-104].

In conclusion, genetic variations may alter the pharmacokinetic and pharmacodynamic profile of certain pharmacologic agents resulting in a difference in drug response. Understanding the molecular/genetic mechanisms of these variations will allow optimal use of pharmacological agents and individualization of drug therapy.

References

1. Leape LL, Brennan TA, Laird N, Lawthers AG, Localio AR, Barnes BA, et al: The nature of adverse events in hospitalized patients. Results of the Harvard medical practice study II. N Engl J Med 1991; 324: 377-384.
2. Jick H: Adverse drug reactions: the magnitude of the problem. J Allergy Clin Immunol 1984; 74: 555-557.
3. Motulsky AG: Drug reactions, enzymes, and biochemical genetics. JAMA 1957; 165: 835-836.
4. Evans WE, Relling MV: Pharmacogenomics: Translating functional genomics into rational therapeutics. Science 1999; 286: 487-491.
5. Διογένης Λαέρτιος: Πυθαγόρας. Vitae Philosophorum, VIII, 8.33.6-8.34.6.
6. Ιπποκράτης: Άπαντα, περί γόνης: 7.16-8.12.
7. Ιπποκράτης: De morbo sacro: 2.7-2.11.
8. Claverie JM: Gene number. What if there are only 30,000 human genes? Science 2001; 291: 1255-1257.
9. Mendel G: Verhandlungen des naturforschenden (Experiments in plant hybridization). Vereines in Brunn, Bd.IV für das Jahr 1865, Abhanlungen, 3-47.
10. Watson JD, Crick FHC: A Structure for Deoxyribose Nucleic Acid. Nature 1953; 171: 737-738.
11. Watson JD, Crick FHC: Genetical Implications of the Structure of the Deoxyribonucleic Acid. Nature 1953; vol: 964-967.
12. Stoneking M: Single nucleotide polymorphisms. From the evolutionary past. Nature 2001; 15: 821-822.
13. Zhou G, Kamahori M, Okano K, Chuan G, Harada K, Kambara H: Quantitative detection of single nucleotide

polymorphisms for a pooled sample by a bioluminometric assay coupled with modified primer extension reactions (BAMPER). Nucleic Acids Res 2001; 29: E93.
14. Jaffe B: Crucibles-The stories of great chemists. The World Publishing Company, Cleveland and New York 1942; pp. 175-198.
15. Garrod AE: Inborn Factors in Disease: An Essay. Oxford Unoversity Press, New York, 1931.
16. Dern RJ, Beutler E, Alving AS: The hemolytic effect of primaquine. J Lab Clin Med 1954; 44: 171-176.
17. Carson PEF, Flanagan CL, Ickes CE, Alving AS: Enzymatic deficiency in primaquine-sensitive erythrocytes. Science 1956; 124: 484-485.
18. Vessel ES, Page JG: Genetic control of drug levels in man: phenylbutazone. Science 1968; 159: 1479-1480.
19. Grand DM: Molecular genetics of the N-acetyltransferases. Pharmacogenetics 1993; 3: 45-50.
20. Hickman D, Sim E: N-Acetyltransferase polymorphism: comparison of phenotype and genotype in humans. Biochem Pharmacol 1991; 42: 1007-1014.
21. Spielberg SP: N-Acetyltransferases: pharmacogenetics and clinical consequences of polymorphic drug metabolism. J Pharmacokinetics Biopharmaceutics 1996; 24: 509-519.
22. Relling MV: Polymorphic drug metabolism. Clin Pharmacol 1989; 8: 852-863.
23. Delomenie C, Sica L, Grant DM, Krishnamoorthy R, Dupret JM: Genotyping of the N-acetyltransferase (NAT2*) gene locus in two native African populations. Pharmacogenetics 1996; 6: 117-185.
24. Cascorbi J, Drakoulis N, Brockmoller J, Maurer A, Sperling K, Roots I: Arylamine N-Acetyltransferase (NAT2) mutations and their allelic linkage in unrelated Caucasian individuals: Correlation with phenotype activity. Am J Hum Genet 1995; 57: 581-592.
25. Okumura K, Kita T, Chikazawa S, Komada F, Iwakawa S, Tanigawara Y: Genotyping of N-acetylation polymorphism and correlation with procainamide metabolism. Clin Pharmacol Ther 1997; 61: 509-517.
26. Perry HM, Tam EM, Carmody S, Sakamoto A: Relationship of acetyl transferase activity to antinuclear antibodies and toxic symptoms in hypertensive patients treated with hydralazine. J Lab Clin Med 1970; 76: 114-125.
27. Takahashi H, Kashima T, Nomizo Y, Muramoto N, Shimizu T, Nasu K, et al: Metabolism of warfarin enantiomers in Japanese patients with heart disease having defferent CYP2C9 and CYP2C19 genotypes. Clin Pharmacol Ther 1998; 63: 519-528.
28. Aithal GP, Day CP, Kesteven PJ, Daly AK: Association of polymorphisms in the cytochrome P450 CYP2C9 with warfarin dose requirement and risk of bleeding complications. Lancet 1998; 353: 717-719.
29. Pianezza ML, Sellers EM, Tyndale RF: Nicotine metabolism defect reduces smoking. Nature 1998; 393: 750.
30. Miyamoto M, Umetsu Y, Dosaka-Akita H, et al: CYP2A6 gene deletion reduces susceptibility to lung cancer. Biochem Biophys res Commun 1999; 261: 658-660.
31. Guyton AC: Blood-pressure control - special role of the kidneys and body fluids. Science 1991; 252: 1813-1816.
32. Gaillard I, Clauser E, Corvol P: Structure of the Human Angiotensinogen Gene; DNA 1989; 8: 87-99.
33. Jeunemaitre X, Soubrier F, Kotelevtsev YV, Lifton RP, Williams CS, Charru A, et al: Molecular basis of human hypertension: role of angiotensinogen. Cell 1992; 71: 169-180.
34. Caulfield M, Lavender P, Farrall M, et al: Linkage of the Angiotensinogen Gene to Essential Hypertension. NEJM 1994; 330: 1629-1633.
35. Hingorani AD, Haiyan J, Stevens PA, Hopper R, Dickerson EC, Brown MJ: Renin-Angiotensin System Gene Polymorphisms Influence Blood Pressure and the Response to Angiotensin Converting Enzyme Inhibition. J Hypertens 1995; 13: 1602-1609.
36. Hunt SC, Cook NR, Oberman A, et al: Is Hypertension Prevention by Sodium Reduction Related to Angiotensinogen Genotype? The Trials of Hypertension Prevention Study, Phase II; Circulation 1997; 96 (Suppl): I-437.
37. Hopkins PN, Lifton RP, hollenberg NK: Blunted renal vascular response to angiotensin II is associated with a common variant of angiotensinogen gene and obesity. J Hypertens 1996; 14: 199-207.
38. Inoue I, Nakajima T, Williams CS, Quakenbush J, Puryear R, Powers M, et al: A nucleotide substitution in the promoter of human angiotensinogen is associated with arterial hypertension and affects basal transcription in vitro. J Clin Invest 1997; 99: 1786-1797.
39. Soubrier F, Hubert C, Testut P, Nadaud S, Alhenc-Gelas F, Corvol P: Molecular biology of the angiotensin I converting enzyme: Biochemistry and structure of the gene. J Hypertens 1993; 11: 471-476.
40. Rigat B, Hubert C, Alhenc-Gelas F, Cambien F, Corvol P, Soubrier F: An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half of the variance of serum enzyme levels. J Clin Invest 1990; 86: 1343-1346.
41. Tiret L, Rigat B, Visvikis S, Breda C, Corvol P, Cambien F, et al: Evidence from combined segregation and linkage analysis, that a variant of the angiotensin-converting enzyme (ACE) gene controls plasma ACE levels. Am J Hum Genet 1992; 51: 197-205.
42. Cambien F, Poirier O, Lecerf L, Evans A, Cambou JP, Arveiler D, et al: Deletion polymorphism in the gene for angiotensin-converting enzyme is a potent risk factor for myocardial infarction. Nature 1992; 359: 641-644.
43. Lindpaintner K, Pfeffer MA, Kreutz R, Stampfer MJ, Grodstein F, LaMotte F, et al: A prospective evaluation of an angiotensin-converting enzyme gene polymorphism and the risk of ischemic heart disease. N Engl J Med 1995; 332: 706-711.
44. Pinto YM, van Gilst WH, Kingma JH, Schunkert H: Deletion-type allele of angiotensin converting enzyme gene is associated with progressive ventricular dilatation after anterior myocardial infarction. Captopril and Thrombolysis Study Investigators. J Am Coll Cardiol 1995; 25: 1622-1626.
45. Schunkert H, Hense HW, Holmer SR, Stender M, Perz S, Keil U, et al: Association between a deletion polymorphism of the angiotensin-converting enzyme gene and left ventricular hypertrophy. N Engl J Med 1994; 330: 1634-1638.
46. Andersson B, Sylven C: The DD genotype of the ACE-gene is associated with increased mortality in idiopathic heart failure. J Am Coll Cardiol 1996; 28: 162-167.
47. Sasaki M, Oki T, Iuchi A, Tabata T, Yamada H, Manabe K, et al: Relation Between the Angiotensin Converting Enzyme Gene Polymorphism and the Effects of Enalapril on Left Ventricular Hypertrophy and Impaired diastolic Filling

in Essential hypertension: M-Mode and Pulsed Doppler Echocardiographic Studies. J Hypertens 1996; 14: 1403-1408.
48. Penno G, Chaturvedi N, Talmud PJ, Cotroneo P, Manto A, Nannipieri: Effect of Angiotensin Converting Enzyme (ACE) Gene Polymorphism on Progression of Renal Disease and the Influence of ACE Inhibition in IDDM Patients. Diabetes 1998; 47: 1507-1511.
49. van Essen GG, Resma PL, de Zeeuw D, Sluiter WJ, Scheffer H, Apperloo de Jong PE: Association between angiotensin-converting - enzyme gene polymorphism and failure of renoprotective therapy. Lancet 1996; 347: 94-95.
50. Hingorani AD, Brown MJ: A simple molecular assay for the C1166 variant of the angiotensin II type 1 receptor gene. Biochem Biophys Res Commun 1995; 213: 725-729.
51. Benetos A, Topouchian J, Ricard S, Gautier S, Bonnardeaux A, Asmar R, et al: Influence of Angiotensin II Type 1 Receptor Gene Polymorphisms on Aortic Stiffness in never treated hypertensive patients. Hypertension 1995; 26: 44-47.
52. Benetos A, Gautier S, Ricard S, Topouchian J, Asmar R, Poirier O, et al: Influence of Angiotensin Converting Enzyme and Angiotensin II Type 1 Receptor Gene Polymorphisms on Aortic Stiffness in Normotensive and Hypertensive Patients. Circulation 1996; 94: 698-703.
53. Benetos A, Cambien F, Gautier S, Ricard S, Safar M, Laurent S, et al: Influence of the Angiotensin II Type 1 Receptor Gene Polymorphism on the Effects of Peridnopril and Nitredipine on Arterial Stiffness in Hypertensive Individuals. Hypertension 1996; 28: 1081-1084.
54. Miller JA, Thai K, Scholey JW: Angiotensin II type 1 receptor gene polymorphism predicts response to losartan and angiotensin II. Kid Int 1999; 56: 2173-2180.
55. Israili ZH, Hall WD: Cough and angioneurotic edema associated with angiotensin-converting enzyme inhibitor therapy. Ann Intern Med 1992; 117: 234-242.
56. Furuya K, Yamaguchi E, Hirabayashi T, Itoh A, Hizawa N, Ohnuma N, et al: Angiotensin-converting - enzyme gene polymorphism and susceptibility to cough. Lancet 1994; 343: 354.
57. Braun A, Kammerer S, Bohme E, Muller B, Roscher AA: Identification of polymorphic sites of the human bradykinine B2 receptor gene. Biochem Biophys Res Commun 1995; 211: 234-240.
58. Mukae S, Aoki S, Itoh S, Iwata T, Ueda H, Katagiri T: Bradykinine B2 Receptor Gene Polymorphism Is Associated With Angiotensin Converting Enzyme Inhibitor-Related Cough. Hypertension 2000; 36: 127-137.
59. Naruse K, Shimizu K, Muramatsu M, Toki Y, Miyazaki Y, Okumura K, et al: Long-term inhibition of NO synthase promotes atherosclerosis in hypercholesterolemic rabbit thoracic aorta: PGH2 does not contribute to impaired endothelium-dependent relaxation. Arterioscler Thromb Vasc Biol 1994; 14: 746-752.
60. Marsden PA, Heng HH, Scherer SW, Stewart RJ, Hall AV, Shi XM, et al: Structure and chromosomal localization of the human constitutive endothelial nitric oxide synthase gene. J Biol Chem 1993; 268: 17478-17488.
61. Lembo G, DeLuca N, Battagli C, Iovino G, Aretini A, Musicco M, et al: A common variant of endothelial nitric oxide synthase (Glu298Asp) is an independent risk factor for carotid atherosclerosis. Stroke 2001; 32: 735-746.
62. Hingorani A, Liang CF, Fatibene J, Lyon A, Monteith S, Parsons A, et al: A common variant of endothelial nitric oxide synthase (Glu298Asp) is a major risk factor for coronary artery disease in the UK. Circulation 1999; 100: 1515-1520.
63. Miyamoto Y, Saito Y, Kajiyama N, Yoshimura M, Shimasaki Y, Nakayama M, et al: Endothelial nitric oxide synthase gene is positively associated with essential hypertension. Hypertension 1998; 32: 3-8.
64. Kato N, Sugiyama T, Morita H, Nabika T, Kurihara T, Yamori Y, et al: Lack of evidence for association between the endothelial nitric oxide synthase and hypertension. Hypertension 1999; 33: 933-936.
65. Jachymova M, Horky K, Bultas J, Kozich V, Jindra A, Peleska J, et al: Association of the Glu298Asp polymorphism in the endothelial nitric oxide synthase gene with essential hypertension resistant to conventional therapy. Biochem Biophys Res Commun 2001; 284: 426-430.
66. Manunta P, Burnier M, D'Amico M, et al: Adducin polymorphism affects renal proximal tubule reabsorption in hypertension. Hypertension 1999; 33: 694-697.
67. Tripodi G, Valtorta F, Torielli L, et al: Hypertension-associated point mutations in the adducing alpha and beta subunits affect actin cytoskeleton and ion transport. J Clin Invest 1996; 97: 2815-2822.
68. Cusi D, Barlassina C, Azzani T, Casari G, Citterio L, Devoto M, et al: Polymorphisms of alpha-adducin and salt sensitivity in patients with essential hypertension. Lancet 1997; 349: 1353-1357.
69. Voilley N, Bassilana F, Mignon C, et al: Cloning, chromosomal localization and physical linkage of the β and γ subunits (SCNN1B and SCNN1G) of the human epithelial amiloride-sensitive sodium channel. Genomics 1995; 28: 560-565.
70. Baker EH, Dong YB, Sangella GA, Rothwell M, Onipinla AK, Markandu ND, et al: Association of hypertension with T594 mutation in β subunit of epithelial sodium channels in black people resident in London. Lancet 1998; 351: 1388-1392.
71. Yang-Feng TL, Xue FY, Zhong WW, Cotecchia S, Frielle T, Canon MG, et al: Chromosomal organization of adrenergic receptors genes. Proc Natl Acad Sci USA 1990; 87: 1516-1520.
72. Borjesson M, Magnusson Y, Hjalmarson A, Andersson B: A novel polymorphism in the gene coding for the beta (1)-adrenergic receptor is associated with survival in patients with heart failure. Eur Heart J 2000; 21: 1853-1858.
73. Humma LM, Puckett BJ, Richasdson HE, et al: Effects of beta1-Adrenoreceptor polymorphisms on resting hemodynamics in patients undergoing diagnostic testing for ischemia. Am J Cardiol 2001; 88: 1034-1037.
74. Liggett SB, Wagoner LE, Craft LL, Hornung RW, Hoit BD, McIntosh TC, et al: The Ile164 β2 adrenergic receptor polymorphism adversely affects the outcome of congestive heart failure. J Clin Invest 1998; 102: 1534-1539.
75. Jia H, Hingorani AD, Sharma P, et al: Association of the Gs alpha gene with essential hypertension and response to beta-blockade. Hypetens 1999; 34: 8-14.
76. Morris BJ: Chromosome 17q23: a locus for cardiovascular disease. Clin Exp Pharmacol Physiol 1993; 20: 279-282.
77. Nurden AT: Polymorphisms of human platelet membrane glycoproteins: structure and clinical significance. Thromb Haemost 1995; 74: 345-351.
78. Weiss EJ, Bray PF, Tayback M, et al: A polymorphism of a platelet glycoprotein receptor as an inherited risk factor for coronary thrombosis. N Engl J Med 1966; 334: 1090-1094.

79. Antiplatelet Trialists' Collaboration: Collaborative overview of randomized trials of antiplatelet therapy-I: prevention of death, myocardial infarction and stroke by prolonged antiplatelet therapy in various categories of patients. BMJ 1994; 308: 81-106.
80. Topol EJ: The future of antiplatelet therapy: optimizing management in patients with acute coronary syndrome. Clin Cardiol 2000; 23(Suppl 6): VI-23-28.
81. Cook GE, Bray PF, Hamlington J, Goldschmidt-Clermont PJ: PlA2 polymorphism and efficacy of aspirin. Lancet 1998; 351: 1253.
82. Michelson AD, Furman, Goldschmidt-Clermont PJ, et al: Platelet GP IIIa PlA polymorphisms display different sensitivities to agonists. Circulation 2000; 101: 1013-1018.
83. Kastrati A, Schoming A, Seyfarth M, et al: Polymorphism of platelet glycoprotein IIIa and the risk of restenosis after coronary stent placement. Circulation 1999; 99: 1005-1010.
84. Walter DH, Schachinger V, Elsner M, Mach S, Dimmeler S, Auch-Schwelk W, et al: Statin therapy is associated with reduced restenosis rates after coronary stent implantation in carriers of the PlA2 allele of the platelet glycoprotein IIIa gene. Eur Heart J 2001; 22: 587-595.
85. Carlsson LE, Santoso S, Baurichter G, et al: Heparin-induced thrombocytopenia: New insights into the impact of FcγRIIa-R-H131 polymorphism. Blood 1998; 92: 1526-1531.
86. The Writing Group for the PEPI Trial: Effects of estrogen or estrogen/progestin regiments on heart disease risk factors in postmenopausal women: The postmenopausal Estrogen/ Progestin Investigation (PEPI) Trial. JAMA 1995; 273: 199-208.
87. Matthews KA, Meilahn E, Kuller LH, et al: Menopause and risk factors for coronary heart disease. N Engl J Med 1989; 321: 641-644.
88. Nakano Y, Oshima T, Matsuura H, Kajiyama G, Kambe M: Effect of 17beta-estradiol on inhibition of platelet aggregation in vitro is mediated by an increase in NO synthesis. Arterioscler Thromb Vasc Biol 1998; 18: 961-967.
89. Hulley S, Grady D, Bush T, et al: Randomized trial of estrogen plus progestin for secondary prevention of coronary heart disease in postmenopausal women. Heart and Estrogen/progestin Replacement Study (HERS) Research Group. JAMA 1998; 280: 605-613.
90. Boudoulas KD, Cook GE, Roos CM, Bray PF, Goldschmidt-Clermont PJ: The PlA polymorphism of Glycoprotein IIIa functions as a modifier for the effect of estrogen on platelet aggregation. Arch Pathol Lab Med 2001; 125: 112-115.
91. Gordon DJ, Rifkind BM: High-density lipoprotein-the clinical implications of recent studies. N Engl J Med 1989; 32: 1311-1316.
92. Kuivenhoven JA, de Knijff P, Boer JMA, et al: Heterogenity at the CETP gene locus: Influence on plasma CETP concentrations and HDL cholesterol levels. Arterioscler Thromb Vasc Biol 1997; 17: 560-568.
93. Zhong S, Sharp DS, Grove JS, et al: Increased coronary heart disease in Japanese-American men with mutation in the cholesteryl-ester transferase protein gene despite increased HDL levels. J Clin Invest 1996; 97: 2917-2923.
94. Kuivenhoven JA, Jukema JW, Zwinderman AH, de Knijff P, McPeterson R, Bruschke AVG, et al: The role of a common variant of the cholesteryl-ester transferase protein gene in the progression of coronary atherosclerosis. N Engl J Med 1998; 338: 86-93.
95. Newby AC, Southgate KM, Davies M: Extracellular matrix degrading metalloproteinases in the pathogenesis of arteriosclerosis. Basic Res Cardiol 1994; 89 Suppl 1: 59-70.
96. Humphries SE, Luong LA, Talmud PJ, Frick MH, Kesaniemi YA, Pasternack A, et al: The 5A/6A polymorphism in the promoter of the stromelysin-1 (MMP-3) gene predicts progression of angiographically determined coronary artery disease in men in the LOCAT gemfibrozil study. Lopid Coronary Angiography Trial. Atherosclerosis. 1998; 139(1): 49-56.
97. Ye S, Watts GF, Mandalia S, Humphries SE, Henney AM: Preliminary report: genetic variation in the human stromelysin promoter is associated with progression of coronary atherosclerosis.Br Heart J 1995; 73(3): 209-215.
98. Ye S, Eriksson P, Hamsten A, Kurkinen M, Humphries SE, Henney AM: Progression of coronary atherosclerosis is associated with a common genetic variant of the human stromelysin-1 promoter which results in reduced gene expression. J Biol Chem. 1996; 31; 271(22): 13055-13060.
99. deMaat MP, Jukema JW, Ye S, Zwinderman AH, Moghaddam PH, Beekr M, et al: Effect of the stromelysin-1 promoter on pravastatin in coronary atherosclerosis and restenosis. Am J Cardiol 1999; 83: 852-856.
100. Emilien G, Ponchon M, Caldas C, Isacson O, Maloteau JM: Impact of genomics on drug discovery and clinical medicin. Q J Med 2000, 93: 391-423.
101. Vessel ES: Advances in Pharmacogenetics and Pharmacogenomics. J Clin Pharmacol 2000; 40: 930-938.
102. March R. Pharmacogenomics: the genomics of drug response. Yeast 2000; 17: 16-21.
103. Kalow W: Perpectives in pharmacogenetics. Arch Pathol Lab Med 2001; 125: 77-80.
104. Watkins RE, Wisely GB, Moore LB, Collins JL, Lambert MH, Williams SP, et al: The human nuclear xenobiotic receptor PXR: Structural determination of directed promiscuity. Science 2001; 292: 2329-2333.