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European Journal of Pharmacology 527 (2005) 1–22
www.elsevier.com/locate/ejphar

Review

Pharmacogenomics and cardiovascular drugs: Need for integrated biological system with phenotypes and proteomic markers

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Received 25 July 2005; received in revised form 23 September 2005; accepted 5 October 2005

Abstract

Personalized medicine is based on a better knowledge of biological variability, considering the important part due to genetics. When trying to identify involved genes and their products in differential cardiovascular drug responses, a five-step strategy is to be followed:

(1) Pharmacokinetic-related genes and phenotypes
(2) Pharmacodynamic targets, genes and products
(3) Cardiovascular diseases and risks depending on specific or large metabolic cycles
(4) Physiological variations of previously identified genes and proteins
(5) Environment influences on them

After summarizing the most well-known genes involved in drug metabolism, we will take as example of drugs, the statins, considered as very important drugs from a Public-Health standpoint, but also for economical reasons. These drugs respond differently in human depending on multiple polymorphisms. We will give examples with common ApoE polymorphisms influencing the hypolipemic effects of statins. These drugs also have pleiotropic effects and decrease inflammatory markers. This illustrates the need to separate clinical diseases phenotypes in specific metabolic pathways, which could propose other classifications, of diseases and related genes.

Hypertension is also a good example of clinical phenotype which should be followed after various therapeutic approaches by genes polymorphisms and proteins markers.

Gene products are under clear environmental expression variations such as age, body mass index and obesity, alcohol, tobacco and dietary interventions which are the first therapeutical actions taken in cardiovascular diseases. But at each of the five steps, within a pharmacoproteomic strategy, we also need to use available information from peptides, proteins and metabolites, which usually are the gene products. A profiling approach, i.e., dealing with genomics, but now also with proteomics, is to be used.

In conclusion, the profiling, as well as the large amount of data, will more than before render necessary an organized interpretation of DNA, RNA as well as proteins variations, both at individual and population level.

– Cluster analyses;
– Multidimensional approaches;
– Pathways and metabolomics;
– Biological systems analyses.

Keywords: ABC transporter; Antiobesity drug; Cardiovascular disease; Cardiovascular drug; Drug-metabolizing enzyme; Drug target; Inflammation; Nutrigenetics; Nutrigenomics; Obesity; Pharmacodynamic; Pharmacogenetic; Statin

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doi:10.1016/j.ejphar.2005.10.011

Contents

1. Introduction

We have recently proposed a five-step pharmacogenomic approach (Siest et al., 2004) for a new cardiovascular drug, successively taking into account the genes involved in:

- pharmacokinetics (absorption, distribution, metabolism, transport),
- pharmacodynamic phase
- specific cardiovascular disorders and related metabolic pathways deviations
- biological variability in genes responses including their physiological regulation, and
- environmental effects.

However, given the current existing pharmacogenomic tools and the development of the pharmacoproteomic field, a complete strategy needs the integration of gene products measured in laboratory medicine. These biomarkers could be the metabolites resulting from drug metabolizing enzymes activities, clusters of peptides related to an inflammatory process, specific proteins or the metabolites resulting from environmental effects. With the extraordinary amount of information which could now be given by the proteins separated from plasma by proteomic devices (HUPO, Van Ommen et al., 2004) (Fathi et al., 2003), we now have to add all these data to the genomic and transcriptomic ones. One DNA molecule is capable in producing a hundred different protein isoforms (Fig. 1).

Biological systems are then the only way to put all this information in a comprehensive and clinically usable set of data (Kitano, 2002). The expression obtained from yeasts will be very useful in preparing a similar approach in human (Ideker et al., 2001; Hall et al., 2004). The genes and their products have to be linked together in metabolic cycle networks which will then be linked to disease entities. In the cardiovascular field, we have e.g. to consider drug metabolism, lipid and lipoproteins metabolism, homocysteine metabolic cycle, renin–angiotensin system, insulin-regulated genes, inflammation markers under expression by specific transcription factors, etc. The gene products are “better” phenotypes than the non-clearly defined disease phenotypes (Bougneres, 2003). We need to organize in this direction the genomic and proteomic information useful for cardiovascular drugs, and to add simultaneously some integrated phenotypes derived from organ imaging, such as carotid intima media thickness or more recent and sophisticated methods.

In addition to our general previous reviews (Siest et al., 2003; Siest et al., 2004), we wrote more specialized ones on pharmacogenomics of antihypertensive drugs (Marteau et al.,2005), hypolipemic drugs (Maumus et al., in preparation) and a review on biological variations of inflammatory markers, cytokines (Berrahmoune et al., 2005).

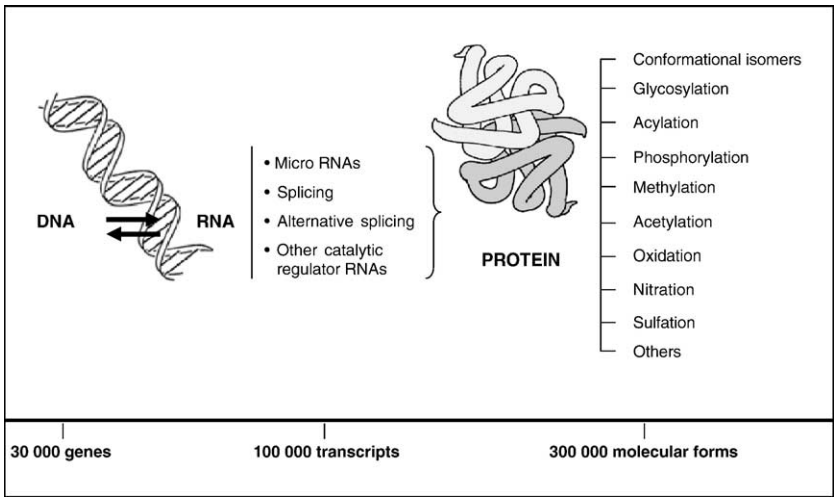

Fig. 1. Increase of information from DNA to RNA and proteins.

We will follow here the same five-point strategy: we have chosen after the drug-metabolizing enzymes involved in cardiovascular drugs metabolism, statins and corresponding pharmacological targets, hypertension-related genes and drugs, inflammation genes and related proteins and peptides, overweight and obesity problems affecting many cardiovascular drug effects. Finally, the future of pharmacoproteomic approach will be discussed.

2. Pharmacokinetics

A large number of cardiovascular drugs are lipophilic and influenced by the four pharmacokinetic processes: absorption, distribution, metabolism, and elimination. Drug-metabolizing enzymes act during either of the two first phases of substrate metabolism: during the functionalization phase (e.g. CYP450) or during the conjugation phase (e.g. glutathione *S*-transferase (GST)). The product is then taken by a transporter (e.g. ATP-binding cassette (ABC) transporters) which eliminates it definitely.

2.1. Genotypes

All genes of these processes are polymorphic. Some examples are presented for phase I (cytochromes *P*450) and phase II (conjugating enzymes) drug-metabolizing enzymes and for phase III drug transporters. For more details, see our last review on pharmacogenomics and drug response in cardiovascular disorders (Siest et al., 2004).

2.1.1. Phase I drug-metabolizing enzymes: cytochromes P450

There are about 60 distinct cytochrome *P*450 (CYP) genes in humans. An up-to-date list of CYP gene polymorphisms can be obtained online (Homepage of the Human Cytochrome *P*450 (CYP) Allele Nomenclature Committee; http://www.imm.ki.se/CYPalleles/). Interestingly, although many of these CYP450 have the capacity to metabolize drugs, the majority of CYP-mediated drug metabolism in humans is catalyzed by the CYP enzyme subfamilies CYP2D6, CYP3A, and CYP2C. Some of these pathways are shown to have consequences on pharmacokinetic of the concerned drug. CYP2D6 is not a major CYP enzyme in terms of quantity in the liver. However, it metabolizes one-quarter of all drugs, and about 40 of them are used in the treatment of cardiovascular diseases. Individuals with geneticall-ly determined low or no CYP2D6 enzyme activity are referred to as poor metabolizers, whereas individuals with fully functional enzyme are known as extensive metabolizers. Poor metabolizers are at increased risk for excessive or prolonged therapeutic effect or toxicity, while ultra-rapid metabolizers may not achieve sufficient therapeutic levels of the drug to be efficient. For example, the clearance of the β-blocker metoprolol is decreased in poor metabolizers, leading to risk of hypotension or bradycardia. About 50 cardiovascular drugs like calcium channel blockers or statins are metabolized by CYP3A family. In addition, about 30 are metabolized by CYP2C family, like some anticoagulants antidiabetics and some non-steroidal anti-inflammatory drugs (NSAIDs) (Siest et al., 2004).

2.1.2. Phase II drug-metabolizing enzymes

Genetic polymorphisms have been described in many phase II drug-metabolizing enzymes. However, they seem to be of less importance to the pharmacogenomics of cardiovascular drugs. The exception may be the glutathione *S*-transferase enzymes which are candidate genes for the modulation of cardiovascular risks. In fact, GSTs are involved in protection of vascular tissue against oxidative stress and cellular damages induced by various pro-atherogenic agents. Inflammation and oxidative stress are two phenomena contributing to cardiovascular pathologies such as atherosclerosis. GSTM1 and GSTT1 polymorphisms could modulate inflammatory risks (Habdous et al., 2004). According to these authors, GSTM1*0 null allele and GSTT1*1 functional allele were associated with increased risk of coronary heart diseases in smokers and GSTT1*1 was associated with increased risk of lower extremity arterial disease.

2.1.3. Phase III drug transporters

Enzymatic metabolism is not the sole determinant of drug pharmacokinetics. Drug transporters, which can be subdivided

Table 1
List of human ABC (ATP-binding cassette) transporters, their functions and diseases caused by ABC genes

Transporter	Functions	Diseases
ABCA1	Cholesterol and phospholipids transport	Tangier disease (homozygous)
	Hypolipidemic drugs: induction (e.g. Clofibrate, Guan et al., 2003) or inhibition (e.g. statins, Ando et al., 2004)	HDL deficiency (heterozygous) Increased risk of atherosclerosis
ABCA2	Sterols transport (brain, macrophages)	Drug resistance
	Cholesterol responsive gene	Links to Alzheimer's disease?
ABCA6, A10	Functions in macrophage lipid homeostasis?	
ABCA7	Phospholipids and cholesterol transport	Sjogren's syndrome?
ABCA9	Functions in macrophage lipid homeostasis and monocyte differentiation?	
ABCB1	Cholesterol and phospholipids transport (minor pathway)	Anticancer drugs resistance
	Hydrophobic xenobiotic compounds transport	Modification of some cardiovascular drugs activity
	Hypolipidemic drugs: inhibition (e.g. fenofibrate and some statins (Ehrhardt et al., 2004)	
ABCB4 (MDR3)	Bile-acid transport Secretion of phosphatidylcholine in bile	Progressive familial intrahepatic cholestasis-3 Cholestasis of pregnancy
ABCB7	Iron transport	X-linked sideroblastosis and anaemia
ABCB11 (BSEP)	Bile-acid transport	Progressive familial intrahepatic cholestasis-2
ABCC1	Leukotrienes transport	Anticancer drugs resistance
ABCC2 (MRP2)	Bile-acid transport	Anticancer drugs resistance Dubin–Johnson Syndrome
ABCC4	Nucleosides transport Prostaglandines transport	Resistance to antiviral and anticancer drugs
ABCC6	Unknown	Pseudoxanthoma elasticum Increased risk of premature coronary artery disease (Trip et al., 2002)
ABCC8 (SUR1)	ATP-sensitive potassium channel subunit in pancreatic cells: modulator of insulin release	Persistent hyperinsulinemic hypoglycaemia of infancy Non-insulin-dependent diabetes mellitus
ABCC9	ATP-sensitive potassium channel subunit in cardiac, skeletal, and vascular and non-vascular smooth muscle	Susceptibility to dilated cardiomyopathy (Bienengraeber et al., 2004)
ABCC10	Lipophilic anions transporter (e.g. estradiol 17â-D-glucuronide)	Anticancer drug resistance (taxanes, Hopper-Borge et al., 2004)
ABCD1	Very long chain fatty acids transport	Adrenoleukodystrophy
ABCG1	Macrophage cholesterol and phospholipid export (Klucken et al., 2000), ABCA1-independent pathway Induction by sterols (Schmitz et al., 2001) and inhibition by some statins (Wong et al., 2004)	–
ABCG4	Promote cholesterol efflux (minor in macrophages)	–
ABCG5, G8	Regulation of intestinal sterols (cholesterol and plant sterols) absorption and hepatic sterols output	Sitosterolemia
	Induction by sterols (Schmitz et al., 2001)	Variation in cholesterol absorption efficiency depending on polymorphisms

Compiled from Stefkova et al., 2004; Efferth, 2003; Borst and Elferink, 2002, the HGNC Gene Family Nomenclature (http://www.gene.ucl.ac.uk/nomenclature/genefamily/abc.html#table1) and Michael Müller (http://nutrigene.4t.com/humanabc.htm).
ABC: ATP-binding cassette; BSEP: bile salt transporter; MDR: multidrug resistance; MRP: multi-drug-resistant protein.

into uptake and efflux systems, have received increased attention for their role in determining drug disposition, intestinal absorption or renal elimination. Among these systems, we can be found the ATP-binding cassette (ABC) transporters subfamily which contains nearly 50 different transporters.

The analysis of ABC transporters expression profiles is of great interest in monitoring drug effects. In fact, ABC transporters are involved in many physiologic processes, such as lipid transport, sterol homeostasis, immune mechanisms and drug transport. Furthermore, they can cause various human inherited diseases.

Many drugs have been shown to be substrates, inducers or inhibitors of drug transporters. For example, ABCB1, also known as P-glycoprotein, transports about 30 cardiovascular drugs like calcium channel blockers and cardiotonics, e.g. digoxin (Siest et al., 2004). Polymorphisms in the gene coding for this protein have been associated with a higher risk of toxicity of digoxin. Moreover, statins may be substrates or inhibitors of these transporters. Mutations in ABC transporter genes and in phenomena, such as induction, inhibition or interaction, can lead to changes in pharmacokinetic cardiovascular drugs and thus to potential interactions (e.g. the calcium channel inhibitor mibefradil, withdrawn from the market due to serious adverse effects, is both a substrate and an inhibitor of CYP3A and ABCB1). ABC transporters also transport endogen products, such as sterols, biliary acids or phospholipids, which are known to be potentially involved in cardiovascular pathologies. It is important to note that concomitant medications and many other environmental factors such as diet, gender, and overall health, can inhibit or induce enzyme or transporters activity.

Table 1 shows a list of the human ABC transporters which are interesting in cardiovascular field, in their functions and diseases they may be implicated in.

Polymorphic activity of metabolism or drug transport proteins is of great interest in the exploration of adverse drug reactions, especially during drug development. Indeed, if an isoform is involved in the metabolism of a new drug candidate, health authorities may request in vivo information on its metabolic pathway(s). If genetic variation in metabolism is reported to be of in vivo clinical significance, then this would alter the

course of drug development and this knowledge could be translated into clinical practice (Pirmohamed and Park, 2003).

2.1.4. DNA chips

Outside phenotyping, genotyping by polymerase chain reaction (PCR) methods are used to predict the metabolizer status. A specific DNA chip is now on the market: Roche Diagnostics' AmpliChip™ CYP450 Genotyping Test. The AmpliChip™ is the first in its class to be cleared by the FDA (December 2004) for the analysis of genes that influence drug metabolism. This test, which is powered by Affymetrix microarray technology, analyses a patient's Cytochrome *P*450 2D6 and 2C19 genotypes (29 polymorphisms and mutations for the *CYP2D6* gene and 2 polymorphisms for the *CYP2C19* gene) from genomic DNA extracted from blood sample. Now, more CYP450 mutations need to be added to this microarray.

Other microarrays are home-made in different laboratories depending on their working thematics. For example, Jaakson et al. (2003) constructed the ABCR400 microarray, a comprehensive mutation detection system for ABCR (ATP-binding transporter retina-specific)-associated retinopathies. This screening tool contains all currently known disease-associated genetic variants and many common polymorphisms of the *ABCR* gene. The ABCR400 microarray offers a prototype diagnostic tool to advance knowledge in ABCR-associated pathology and in ophthalmic genetics generally.

2.2. Phenotypes

If it is not possible to measure directly the protein or the RNA, proteic activity can be estimated by dosing one of the exo/endogenous substrates of the enzyme/transporter in biological liquids. Thus, as an alternative to genotyping methods, the metabolizer status can be safely determined, using a specific substrate having a large therapeutic index: for example, dextromethorphan or debrisoquine for CYP2D6. Estradiol 17-β-D-glucuronide is a prototypical glucuronate conjugate which is selected as a model test compounds. Like the glutathione conjugate, leukotriene C4 (LTC4), it can be used radiolabeled to study the transport capacity of the transporters of the multidrug resistance family (Chen et al., 2003).

The cytochromes *P*450 are localized in membranes and these proteins cannot be measured readily in plasma. Sulfotransferases and some glutathione-*S*-transferases are measurable in blood. Overall, measurements of drugs or endogenous metabolites are necessary to evaluate the consequences of the genetic polymorphisms.

Nevertheless, blood cells could sometimes be used to analyse mRNA and proteins corresponding to some enzymes/transporters: CYP2E1 and CYP4B1 mRNA levels in peripheral blood leucocytes reflect activity in the liver and could be measured by Reverse transcriptase (RT)–PCR method (Raucy et al., 2004).

DNA microarray technique has the sensitivity and the selectivity for applications in pharmaceutical sciences and for characterizing the expression of cytochrome *P*450 enzyme mRNAs, other drug-metabolizing enzyme mRNAs, and transporter mRNAs in a variety of clinical samples. The results obtained are corroborated by quantitative real-time reverse transcription–PCR and are generally always confirmed. As the microarray allows the determination of the expression profile of many ABC transporters in a single hybridization experiment, it may be useful as a diagnostic tool to detect drug resistance in clinical samples and for monitoring expression profiles in clinical biopsies and their correlation to clinical treatment. Many ABC transporters microarrays are actually developed. Unfortunately, they are essentially developed in cancer field and the concerned ABC transporters and polymorphisms are not always the ones of interest in the cardiovascular field.

2.2.1. Proteins

Western Blot could be used to quantify some CYP450 or transporters. For example, some anti ABCA1 and Anti ABCB1 antibodies are commercialized. Some others can be created when necessary. However, it is difficult to determine if the protein quantity is representative of the activity.

2.2.2. Substrates

The 6β-hydroxycortisol ratio determination is thus validated for CYP3A4 induction survey in an individual being its own control, while it is still under debate for inhibition survey. This test may not be used as reflecting the basal level of expression of CYP3A4. In fact, some limitations appeared in the use of 6β-hydroxycortisol ratio to measure CYP3A4 activity. Cortisol is a substrate for both CYP3A4 and CYP3A5. The contribution of CYP3A5 (expressed abundantly in the liver and small intestine, but only in 30% of white subjects and 70% of blacks) on the metabolism of 6β-hydroxycortisol is poorly understood. CYP3A4 and CYP3A5 are highly polymorphic with some alleles (e.g. CYP3A5*3) coding for an enzyme with severely decreased activity. The genetic component is thus likely to contribute significantly to the variability of the 6β-hydroxycortisol ratio and such variability is used to evaluate CYP3A induction or inhibition in a given ethnic population (Yin et al., 2004).

The efficiency of membrane transporters can also be assessed by evaluating the ability to accumulate and to extrude exogenous substrates. For example, calcein is already known to be transported by the multi-drug resistance system and in particular by multi-drug resistant protein (MRP) 1 and P-glycoprotein. Exogenous substrates can also be used to detect potent inhibitors, which may also be potent substrates, of a transporter. As an example, the ABCB1 fluorescent substrate, rhodamine 123, is commonly used to investigate the activity of P-glycoprotein inhibitors which affect the uptake of rhodamine 123.

As they are easily available in biological liquids, endogenous substrates are more interesting than exogenous substrates. Table 2 presents the known endogenous substrates and metabolites of various enzymes and ABC transporters of particular interest in cardiovascular field. Depending on their localization and on their capacities to be substrates of others proteins or to

Table 2
Endogenous substrates as potential markers of human cytochrome *P*450 (CYP), UDP-galactose (UGT) transporters and ABC (ATP-binding cassette) transporters

Proteins	Phenotypes (endogenous substrates, metabolites)	References
CYP1A1	Acid arachidonic	
CYP3A4	6β-hydroxy cortisol/cortisol (urine; interest+++ (e.g. monitor CYP3A4 induction))	
CYP7A1	Synthesis of bile salts from cholesterol in the liver	
UGT1A1	Bilirubin, estrogens (estradiol, 17α-ethinylestradiol)	
UGT1A3	Leukotriene B4	
UGT2B4	Bile acids, catechol, estrogens	
UGT2B7	Androgens, biliary acids, estrogens, leukotriene B4, linoleic acid, pregnanes, retinoic acids	
UGT2B10, 2B11	13-Hydroxyoctadecadienoic acid hydroxy-eicosatetraenoic	
UGT2B15	Androgens (dihydrotestosterone), 12-hydroxy-eicosatetraenoic	
UGT2B17	13-hydroxyoctadecadienoic acid	
UGT2B28	Steroids, bile acids	
ABCA1	Unesterified cholesterol (interest++, plasma)	
	Phospholipids (mostly phosphatidylcholine)	Oram et al., 2001
	Vitamin E, apolipoprotein A1 (not substrate, interest +++, plasma)	Tregouet et al., 2004
ABCA6, A8, A9, A10	Unknown	
ABCA7	Cholesterol, phospholipids	
ABCB1 (MDR1)	Cholesterol	
	Phospholipids (phosphatidylcholine)	Romiti et al., 2002
	Sphingomyelin	Romiti et al., 2002
	Platelet-activating factor (PAF)	Romiti et al., 2002
	Sex-steroid hormones like estradiol	Romiti et al., 2002
	Flavonoids	Romiti et al., 2002
	Aldosterone, prednisolone, glucurosylceramide	Romiti et al., 2002
	Bioactive peptides such as somatostatin and substance P	Borst and Elferink, 2002 Uchiyama-Kokubu et al., 2004
ABCB4	Bile acids, phospholipids	
ABCB11 (BSEP)	Bile acids	
ABCC1 (MRP1)	Conjugates of steroid hormones, i.e. oestradiol 17-β-D-glucuronide, DHEAs	Zelcer et al., 2003
	Glutathione-conjugated leukotrienes	Reid et al., 2003
ABCC2 (MRP2)	Bilirubin-glucuronides	Borst et al., 2000
	Estradiol 17-β-D-glucuronide	Zelcer et al., 2003
	Glutathione-conjugated leukotrienes (Leukotriene C4)	Reid et al., 2003
ABCC3 (MRP3)	Glutathione and glucuronate conjugates	Chen et al., 2003
	Monoanionic bile acid	
ABCC4 (MRP4)	Conjugates of steroid hormones, i.e. estradiol 17-β-D-glucuronide, DHEAs	Zelcer et al., 2003
	Bile acids	
	PGE1 and PGE2 and PGF2α, PGA1, and thromboxane B2 presumably substrates, (NSAIDs: inhibitory effect)	Reid et al., 2003
ABCC6 (MRP6)	Glutathione conjugate	Chen et al., 2003
	Desmosines (not substrates, markers of elastin degradation, urine and plasma)	Annovazzi et al., 2004
	Sulfated glycosaminoglycans (not substrates, urine)	Maccari et al., 2003
ABCC10	Estradiol 17-β-D-glucuronide	Chen et al., 2003
ABCG1 (White1, ABC8)	Cholesterol and phospholipids	Klucken et al., 2000
ABCG2 (BCRP)	Sulfated estrogens	Imai et al., 2004
	Phytoestrogens/flavonoids	
ABCG5	Plant sterols (++? plasma) e.g., campesterol and sitosterol	Sudhop and von Bergmann, 2004
ABCG8	Ratio plant sterols to cholesterol?	Gylling et al., 2004
	Serum cholestanol-to-cholesterol ratio: surrogate marker of cholesterol absorption efficiency	
	Cholesterol precursor, e.g. lathosterol, desmosterol, cholestanol	
	Cholesterol	
	Biliary acids	

DHEA: dihydro-epi-androstérone; MRP: multi-drug resistant protein; NSAID: non-steroidal anti-inflamatory drug; PG: prostaglandin.
Interest ++: of a great interest.
Interest +++: of a very great interest.

be influenced by multiple polymorphic genes, some of these phenotypes might be used as potent markers for an enzyme/transporter activity. Thus, they could be measured in biological products like plasma or urines, reference values could then be established and the function of the enzyme/transporter evaluated. However, it is difficult to find a substrate with enough specificity for a protein/transporter to use it like a marker of the protein activity.

6β-Hydroxycortisol urinary excretion was, for a long time, considered as a marker of induction and, more recently, of drug inhibition in humans (Galteau and Shamsa, 2003). But its specificity is still under debate. Most authors consider that 6β-hydroxycortisol ratio is a useful non-invasive test to evaluate inducing properties of drugs towards CYP3A4. The great advantage of this test is that there is no drug to be administered, as cortisol and 6β-hydroxycortisol are natural metabolites.

To our knowledge, none of the transporter substrates are used in everyday practice to measure activity. However, in research laboratories, some substrates are often used, like oestradiol 17-β-glucuronide. Dosage methods are numerous, such as mass spectrometry for prostaglandins.

ABCB1 is well known because of its involvement in drug (in particular, anticancer drugs) resistance. It can also be implicated in modification of pharmacokinetic of cardiovascular drugs transport as it transports some antiarythmics, antihypertensives, cardiotonics or hypolipemics. Its physiological substrates are phospholipids and cholesterol. However, it is a minor pathway in the whole transport of these physiologic substrates.

Quantification of ABCB1 is thus interesting in many studies related to monitoring of drug treatment and of drug interactions with CYP3A substrates for example.

MRPs share overlapping substrate specificity despite different patterns of tissue distribution, localization in polarized cells and protein size. A common feature of MRPs is that they transport a wide variety of organic anions and compounds that are conjugated with sulfate, glucuronate, or glutathione (Zelcer et al., 2003). The oestradiol metabolite oestradiol 17-β-D-glucuronide (E17βG) is a prototypical glucuronate conjugate which is selected as a model test compound to determine the capacity of a multidrug resistance family transporter to be a lipophilic anion pump and a component of the energy-dependent efflux system involved in the cellular extrusion of lipophilic compounds that are metabolized by the covalent attachment of bulky anionic moieties (Chen et al., 2003). This organic anion, which is often used to study transport by MRPs, is transported by MRP1±4 with comparable affinities (Zelcer et al., 2003). However, this substrate can be similarly metabolized by a family of transporters. For example, oestradiol 17-β-D-glucuronide is not really specific as it is transported by all the multidrug resistance. So, we understand that it is difficult to find a specific endogenous marker (substrate or metabolite) for an ABC transporter. When studying phenotype, we cannot overcome the potential influence of another gene. Thus, it will be interesting to consider both the genotype and phenotype of a drug transporter while studying its expression.

3. Pharmacodynamics. Example of statins

In the context of pharmacogenomics and pharmacoproteomics, the evaluation of variations in gene sequence (genetic polymorphisms) and in gene products (concentrations and/or activities) of pharmacological targets is a task important to achieve. There are several pharmacological targets including receptors, enzymes, ion channels, lipoproteins, coagulation factors and signal transduction pathways. These targets may present variations within their gene sequence (e.g. single nucleotide polymorphisms or SNPs), that can alter the effect of the administered drug. We previously reviewed the main described

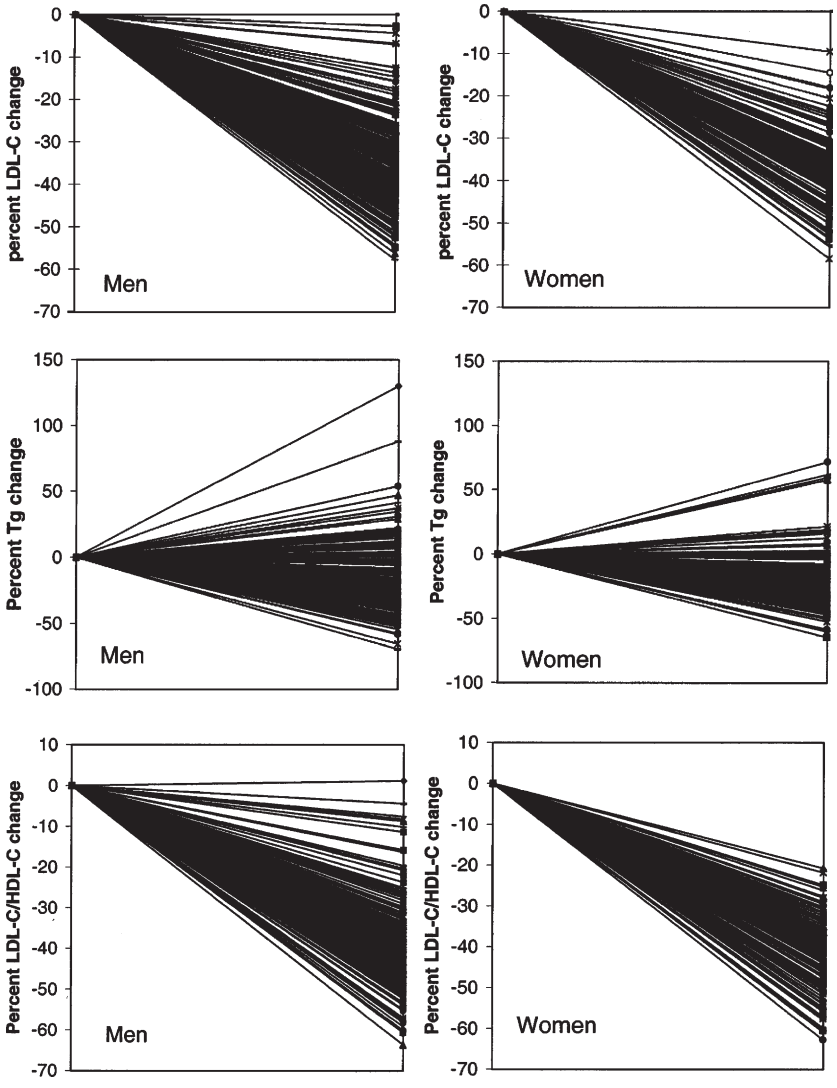

Fig. 2. Individual LDL-cholesterol, triglycerides, and HDL-cholesterol percent response to 10 mg per day of atorvastatin (from Pedro-Botet et al., 2001; with permission).

polymorphisms influencing pharmacodynamics of cardiovascular drugs (Siest et al., 2004). These polymorphisms concern the following genes: α-*adducin* (Gly460Trp), *angiotensinogen* (Met235Thr), *angiotensin-II receptor type 1* (A1166C), *angiotensin-converting enzyme (ACE)* (insertion/deletion (*I/D*)), *cholesteryl ester transfer protein (CETP)* (B1/B2), *apolipoprotein E* (*APOE*) (E2/E4), *factor V Leiden* (Arg506Gln), and the *glycoprotein IIIa (GPIIIa)* (*$Pl^{A1/A2}$ polymorphism*) gene.

In order to illustrate the pharmacodynamic aspect of drug response variability in our current review, we chose the example of pharmacological targets of 3-hydroxy-3-methyl-glutaryl coenzyme A reductase (HMG CoA reductase) inhibitors also called statins. An excellent example of the large range of drug responses induced by statins that can be found in individuals is given by the results of the study of Pedro-Botet et al. (2001) (Fig. 2). Their study investigated 328 men and women who participated in a multicentric, double-blind clinical trial and were treated by atorvastatin at the dosage of 10 mg/day. As shown in Fig. 2, the major part of individuals presented a mean percentage change in low-density lipoprotein (LDL)-cholesterol, triglycerides and high-density lipoprotein (HDL)-cholesterol relatively consistent. However, several patients showed a large interindividual variability that produced a broad range of response. Genetics contribution to the variability of drug disposition and effects is estimated to 20–95% (Evans and McLeod, 2003).

Statins are metabolized by different *P*450 cytochromes, as well as phase II enzymes and their cellular transport is regulated by several transporters. Considering CYPs, 3A4, 3A5, 2C8, 2C9, and 2D6 have variations in their gene that can influence response to statin treatment. Candidate genes that are important to consider for ABC transporters are A1, G5, G8, B1, C2, MCT4, OATP2. According to Schmitz et al. (2001), *ABCG1* facilitates the translocation of phospholipids and cholesterol to the plasma membrane where ABCA1-facilitated efflux mechanisms are active in association with specialized lipid microdomains. Alternatively, *ABCG1* could participate in ABCA1-independent efflux pathways, for example, via apoE or passive diffusion mechanisms. Schmitz et al. (2001) propose that uptake of sterols (LXR ligands) and concomitant activation of the nuclear receptor pathway via LXR/RXR causes transcriptional induction of *ABCG1* and *ABCA1*. Statins regulate the LXR target genes, like *ABCA1* and *ABCG1* (Wong et al., 2004). They may down-regulate cholesterol efflux from non-loaded human macrophages by inhibiting synthesis of an oxysterol ligand for LXR.

More detailed information can be found in a review entirely dedicated to hypolipemic drugs (Maumus et al., submitted for publication). For this specific review, we focused on the effective pharmacological targets of these hypolipemics.

Statins act by affecting cholesterol synthesis, through the competitive inhibition of HMG-CoA reductase, the rate-limiting enzyme in cholesterol biosynthesis. The resulting intracellular reduction of cholesterol concentration induces the activation of SREBP (sterol responsive element-binding proteins) processing proteins namely SCAP (SREBP cleavage activating protein), SP1 and SP2 (site-1 and site-2 proteases). These proteins act synergistically to release the amino-terminal domain of SREBPs (SREBP-1 and -2) by proteolysis. The active domains are then transferred into the nucleus where they up-regulate several genes involved in cholesterol homeostasis, one of which being LDL-receptor gene (Brown and Goldstein, 1999). Recently, two more proteins, namely INSIG-1 and -2, were found to be involved in cholesterol homeostasis regulation by controlling SCAP activation according to local concentrations of cholesterol (Yang et al., 2002; Yabe et al., 2002). Statin therapy was also shown to induce an increase in HDL-cholesterol concentration via a PPAR-α (peroxisome proliferator-activated receptor alpha)-dependent mechanism (Martin et al., 2001; Schaefer et al., 1999; Gaw, 2003).

The therapeutic potential of statins goes further hypolipemic properties as shown by pleiotropic effects exerted by this drug class. Indeed, by altering isoprenylation which in turn induces the inhibition of small GTP-binding proteins Rho, Ras and Rac, statins are able to improve the endothelial function, to enhance the stability of atherosclerotic plaques, to decrease oxidative stress and inflammation, inhibit the thrombogenic response, and to have beneficial effects on immune system (Liao and Laufs, 2005).

When considering Pharmacodynamics for integrative Pharmacogenomics studies, two major biological systems can be assessed, namely target genes (specific and pleiotropic ones), and gene products (lipoproteins, integrative metabolites and proteins assessed for therapeutic surveillance).

3.1. Target genes, or the study of the genotype influence on treatment response

3.1.1. Specific target genes

By the term *target genes*, we refer to the genes which belong either to the mechanism of action or more generally to the metabolic pathway aimed by the drug. Therefore, in our example we will consider target genes of hypolipemic drugs, and more specifically target genes of statins. Here, we deal with genes of lipid pathway implicated directly/indirectly in the mechanism of action of statins.

In addition to genes that are classically described when talking about statins (for example *CETP* and *APOE* genes), recent genes have been recently reported to exhibit polymorphisms that influence the response to this class of drugs. A first list was given by Chasman et al. (2004) which we have tried to complete in a recent review (Maumus et al., in preparation). The list containing the candidate genes for pharmacogenomics of statin is shown in Table 3. The 3-hydroxy-3-methyl-glutaryl coenzyme A reductase gene (*HMGCR*) can be cited as an example of these recently reported polymorphisms. Recently, two common and tightly linked SNPs in the *HMGCR* gene (a A>T substitution at position 74726928 and a T>G substitution at position 74739571) were related to response to pravastatin treatment (Chasman et al., 2004). Individuals with a single copy of the minor allele of these SNPs had their overall efficacy for modifying total cholesterol concentration reduced of 22%. These effects were largely due to differences in LDL cholesterol: individuals heterozygous for the SNP experienced approximate

Table 3
Candidate genes and their respective encoded proteins for an integrative study of the pharmacogenomics of statins

HUGO Gene Nomenclature of candidate genes involved in the lipid pathway (cholesterol synthesis, absorption, transport...) that may affect hypolipemic drugs efficacy by modifying lipid concentration regulation
HMGCR (3-hydroxy-3-methylglutaryl coenzyme A reductase)
FDFT1 (farnesyldiphosphate farnesyltransferase 1, squalene synthase)
LPL (lipoprotein lipase)
HL/LIPC/LIPH (hepatic triglyceride lipase)
EL (endothelial lipase)
PPARA (peroxisome proliferator-activated receptor alpha)
APOA1 (apolipoprotein A-I)
APOA2 (apolipoprotein A-II)
APOC3 (apolipoprotein C-III)
APOA4 (apolipoprotein A-IV)
APO A1-C3-A4 cluster
APOA5 (apolipoprotein A-V)
APOB (apolipoprotein B)
APOE (apolipoprotein E)
Lp(a) (lipoprotein (a))
LCAT (lecithin cholesterol acyltransferase)
CETP (cholesteryl ester transfer protein)
FABPL/FABP1 (liver fatty acid-binding protein)
LDLR (LDL receptor)
FATP (fatty acid transport protein)
SREBP1 (sterol regulatory element-binding protein 1)
SREBP2 (sterol regulatory element-binding protein 2)
SRB1/CLA1
INSIG1 (insulin-induced gene 1)
INSIG2 (insulin-induced gene 2)
SCAP (SREBP cleavage activating protein)
S1P (site-1 protease)
S2P (site-2 protease)
LEPR (leptin receptor)
PON1 (paraoxonase 1)
ACAT/ACAT1 (mitochondrial acetyl-CoA acetyltransferase)
Candidate genes not involved in the lipid pathway
ACE (angiotensin-converting enzyme)
FGB (β-fibrinogen)
MMP3/STMY1 (matrix metalloproteinase 3, stromelysin 1)
GP IIIa (glycoprotein IIIa)
Cd36/GP IIIb (Cd36 antigen, glycoprotein 3b, fatty acid translocase)
ESR1 (estrogen receptor α)

19% smaller LDL-cholesterol reduction after pravastatin treatment. On the contrary, no significant difference was found between genotypes concerning the change in HDL-cholesterol with pravastatin. The differences observed in total cholesterol reduction by genotype were also true when studying men and women separately, even if these were more significant in men.

3.1.2. Pleiotropic target genes

These genes, which concern other metabolic pathway, have generally been proposed previously to be candidate to cardiovascular diseases. The pleiotropic genes which variations have been studied with statins are the angiotensin-converting enzyme (*ACE*) gene (Bray et al., 2001), the β-fibrinogen (*FGB*) gene (De Maat et al., 1998), the glycoprotein IIIa (*GP IIIa*) gene (Bray et al., 2001), the stromelysin-1 (*MMP3*) gene (De Maat et al., 1999), the *CD36* gene (Nakamura et al., 2004), and the estrogen receptor alpha (*ESR1*) gene (Kajinami et al., 2005).

A recent example of the pleiotropic target genes that can alter the lipid response to statin was given by Kajinami et al. (2005). The *ESR1 Pvu*II(−) *Xba*I(+) haplotype was significantly and independently associated with a greater HDL-cholesterol raising in women, but not in men, in 338 hypercholesterolemic patients treated by atorvastatin. Thus, the estrogen receptor-mediated pathway may play a role in HDL-cholesterol response to statin treatment (Kajinami et al., 2005).

3.2. Gene products

Here we describe products deriving from the target genes which can be assessed as proteomic markers. In the case of statins, the first gene products to study are unequivocally lipids. An absolute list of the gene products that would be interesting to study in the case of statins is given in Table 3. In clinical practice, the first gene products to study are unequivocally lipids. However, in addition to the classical determination of triglycerides, total cholesterol, HDL-cholesterol and LDL-cholesterol levels, the assessment of other lipoproteins plus apolipoproteins could be done.

3.2.1. Lipoproteins

Lipoprotein and apolipoprotein assessments can be managed to follow the efficacy of a given treatment. The lipoproteins that could be surveyed are HDL particles, LDL particles, intermediate-density lipoprotein (IDL) particles, very-low density lipoprotein (VLDL) particles, and chylomicrons.

Of course, the assessment of these various constituents can bring valuable information only if considering that the proteins in question may present biological variations (as well as genetic ones). Thus, thanks to the STANISLAS cohort study (Siest et al., 1998; Mansour-Chemaly et al., 2002), we were able to propose reference values for the proteins described above, taking into account biological and genetic variations (Siest et al., 1990; Vincent-Viry et al., 1998; Tilly et al., 2003).

3.2.2. Modified proteins

Like glycated hemoglobin in diabetes survey, circulating oxidized proteins and in particular oxidized LDL (Ox-LDL) could serve as biomarkers of exposition in hypercholesterolemia management, especially for surveying statin-treated patients. Elevated levels of plasma Ox-LDL were associated with the existence of coronary heart disease (Toshima et al., 2000) and the severity of acute coronary syndromes (Ehara et al., 2001). Fluvastatin has radical scavenging activity and is effective in the oxidative modification of LDL-cholesterol (Hussein et al., 1997; Suzumura et al., 1999). Recently, Inami and coworkers have shown for the first time that the level of circulating Ox-LDL was significantly decreased by stain treatment. Additionally, fluvastatin was more effective than pravastatin with regard to Ox-LDL decrease (Inami et al., 2004).

3.2.3. Protein assessed for therapeutic surveillance

These biomarkers are currently used for surveying drug side effects. Statins are a good example to illustrate this part. Indeed,

in some cases statins can cause elevation of creatine phosphokinase (CPK) which signs the appearance of muscular lysis (rhabdomyolysis), the major side effects caused by statins. CPK are currently surveyed in statin treatment. The second major side effect caused by statins is an alteration of the hepatic function, which is surveyed by the assessment of transaminases levels. Transaminases control is imperative at least once in the three months following the beginning of treatment.

The mechanism by which statins induce increase in CPK levels has remained unclear for a long time. Recently, it was suggested that the modulation of isoprenoid-dependant processes may be involved in the pathophysiology of statin-related myopathy (Schmitz and Drobnik, 2003).

The first quality required for a biomarker checking that the treatment is evolving without adverse drug effects is to be easily assessed in routine, because the dosage concerns a great part of patients treated by the drug and because the assessment must be done regularly. Although very useful in clinical practice, one drawback of CPK is their lack of specificity due to their high sensitivity to physical activity and their great interindividual variability.

3.2.4. Integrated phenotypes derived from imaging studies

The first stage in atherosclerotic lesion formation is a thickening of the intima that results from proliferation of smooth muscle cells. Measurement of carotid intima-media thickness (IMT) by B-mode ultrasound is a well-validated procedure of tracking atherosclerosis progression and detecting early lesions. In their review, Grobbee and Bots examine the evidence for imaging studies for the efficacy of statins in slowing atherosclerosis progression and promoting regression of disease (Grobbee and Bots, 2004). These authors summarize the effects of statin therapy on the progression of atherosclerosis which have been examined in several clinical trials using vascular image techniques. Among their conclusions, they establish that carotid IMT determines plaque size directly rather than estimating plaque burden on the basis of luminal stenosis and, therefore, it may be a more sensitive technique for detecting subclinical atherosclerosis and lipid-rich plaques that are vulnerable to rupture.

At least 30% of patients with dyslipidemias are also hypertensive. Therefore, logically, while we chose to illustrate the variability in the drug response caused by pharmacodynamics with the example of hypolipemic statins, a good example of pathologic condition that may induce drug response variability is hypertension.

4. Hypertension: genes and protein markers

Hypertension is a multi-factorial disorder that likely results from the inheritance of a number of susceptibility genes and involves multiple environmental determinants. It is difficult to predict if an antihypertensive drug will be effective in patient. However, genetic approach based on the attempt to relate candidate-genes to antihypertensive drug effects was proposed in order to improve this responsiveness.

Lot of polymorphisms in genes of the renin–angiotensin system (RAS) or salt regulation system, are associated with blood pressure regulation. Few variants are related to protein expression and several are currently described to influence blood pressure (BP)-response to antihypertensive drugs (Table 4). In the case of hypertension, the choice of the endpoint i.e. blood pressure decrease needs to be seen in addition to measurements including gene products and metabolites. Indeed, evaluation of these products could be useful in the concern of adverse effects that are not enough taken into consideration in usual pharmacogenetic studies. To illustrate this concept, only few major polymorphisms that could predict BP-response to antihypertensive drugs are described.

4.1. Polymorphisms, metabolites and antihypertensive drugs

The *ACE* insertion/deletion (I/D) polymorphism is one of the most famous polymorphism of the RAS known that is linked to hypertension. The deletion allele of the polymorphism is strongly associated with an increased level of circulating ACE (Rigat et al., 1990) and serum ACE activity has been recently correlated with measured adherence with ACE inhibitor treatment in congestive heart failure (Struthers et al., 2001) suggesting that more investigations should be done in other diseases such as hypertension.

Methods to detect therapeutic concentrations of ACE inhibitors, such as benazepril, enalapril, perindopril, quinapril, ramipril, trandolapril, their metabolites, or both, in human samples have been drawn (Maurer et al., 1998). Results have led to show that trandolapril was more active than enalapril on blood pressure and ACE activity, respectively and that its active metabolite (trandolaprilat) had greater lipophilicity and a better affinity to ACE (Chevillard et al., 1994). Surprisingly, although *ACE* I/D polymorphism was described to have an influence on the ACE inhibitor response, it does not seem to influence the adherence to these drugs (Schelleman et al., 2005). In this case, serum ACE activity could be more predictive to ACE inhibitor response than *ACE* I/D polymorphism. This suggestion is emphasized by recent findings suggesting that a common factor is involved in the regulation of serum ACE, which is different from the genetic determination of ACE activity (Boomsma et al., 2005). Nevertheless, if high doses of captopril and ACE inhibitors could inhibit ACE activity, the level of inhibition is not correlated with neither vascular pharmacodynamic performs nor clinical improvement. In particular, low doses of ACE inhibitors per os decrease ACE activity transitorily while efficacy increases with time. Another negative argument in the proposition of ACE activity as direct risk factor is that ACE substrates, i.e. angiotensin I and bradykinin, seem to be the main factors that limit enzymatic reaction and that ACE anchored in endothelial membrane could be more important in terms of activity than circulating enzyme. The bradykinin half-life is too short to consider the relevance of bradykinin levels measurements in determining ACE inhibitor efficacy. The use of synthetic substrates (furylacryliques peptides) is also currently preferred than these two endogenous substrates in order to evaluate ACE inhibitor treatment efficacy and cardiovascular risk factor. However, the field of metabolic pathway activity is of a particular interest to rapidly and accurately identify the

Table 4
Genes/polymorphisms and predictive BP response to antihypertensive drugs

Gene	SNP	Effect(s)	BP response to antihypertensives	References
α-Adducin (ADD1)	Gly460Trp	↘ Renin level ↘ Erythrocyte sodium content and faster Na–K cotransport	Greater BP reduction in response to diuretics but need to be confirmed	Sciarrone et al., 2003; Turner et al., 2003
α_2-Adrenoceptor (ADRA2)	1817 G/A 278 G/T		Greater BP reduction in response to beta-blockers	Hingorani et al., 1995; Liljedahl et al., 2003
Angiotensinogen (AGT)	Met235Thr	Association with blood pressure	Greater BP reduction possible in response to ACE inhibitor, β-blockers, A-II receptor blockers	Liljedahl et al., 2003; Kurland et al., 2004
	Thr174Met		No association in response to A-II receptor blocker — possible BP reduction in response to β_1-blocker	Kurland et al., 2002; Kurland et al., 2004
	−20A/C		No association in response to β_1-blocker, A-II receptor blocker	Kurland et al., 2004
	−6G/A	AGT levels ↗	Greater BP reduction in response to β_1-blocker and diuretic but not A-II receptor blocker	Kurland et al., 2004
Angiotensin-converting enzyme (ACE)	I/D	ACE levels ↗ ↗ Risk of developing CAD	Insertion allele could predict BP reduction in response to ACE inhibitor, A-II receptor blocker, diuretic — no BP response to â1-blocker, Ca^{2+} channel blocker	Sciarrone et al., 2003; Liljedahl et al., 2003; Schwartz et al., 2002; Nakano et al., 1997
	12 557A/G		Greater BP reduction in response to A-II receptor blockers	Liljedahl et al., 2003; Kurland et al., 2004
Angiotensin II receptor type I (AGTR1)	1116A/C	Association with hypertension is controversial	Greater BP reduction in response to ACE inhibitors and diuretic possible	Hingorani et al., 1995; Kurland et al., 2001; Frazier et al., 2004
Aldosterone synthase (CYP11B2)	−344C/T	T allele seems to be more associated with higher aldosterone excretion and blood pressure than C allele	Greater BP reduction in response to A-II receptor blocker but not diuretic and β-blocker	Liljedahl et al., 2003; Kurland et al., 2002; Frazier et al., 2004; Ortlepp et al., 2002; White and Rainey, 2005
G-protein α-subunit (GNAS1)	FokI (+/−) ATT→ATC	Association with hypertension	*FokI*$^+$ allele associated with a greater reduction of BP in response to β_1-blocker	Jia et al., 1999
G-protein β_3-subunit (GNB3)	825C/T	Lower renin levels, higher aldosterone to renin ratio	Greater BP reduction in response to diuretic and α_2-adrenoceptor agonist	Turner et al., 2001; Nurnberger et al., 2003
β_1-Adrenoceptor (ADRB1)	Arg389Gly		Greater BP reduction in response to β_1-blockers	Turner et al., 2003; Johnson et al., 2003; O'Shaughnessy et al., 2000
β_2-Adrenoceptor (ADRB2)	1309G/A and 1342G/C Gly16 Gln27	Confer an ↗ risk for stage-2 hypertension Cardiac hypertrophy	1309G/A and 1342G/C associated with a greater BP reduction in response to β_1-blockers. Gly16 and Gln27 alleles are not predictive of BP-response to diuretic or β-blockers, respectively	Turner et al., 2003; Liljedahl et al., 2003; Jia et al., 2000
Endothelin receptor type B (EDNRB)	40G/A	High circulating plasma levels of endothelin reported in hypertension	Frequent allele associated with a BP reduction in response to β_1-blockers and A-II receptor blocker	Liljedahl et al., 2003

BP: blood pressure; CAD: coronary artery disease.

genetic determinants of adrenoceptors (ADRs) to ACE inhibitor medication. Indeed serum amyloid protein can predict susceptibility to ACE inhibitor-associated angiodema and it is possible to measure serum acute phase proteins activity using substrates that mimic des-Arg^9-bradykinin.

As with the ACE locus, several publications aim to predict patient response to antihypertensive drugs using genetic polymorphisms at the *angiotensinogen* locus (AGT). Carriers of the T allele genotyped for the *angiotensinogen* Met235Thr variation tend to have higher plasma levels of angiotensinogen (Jeunemaitre et al., 1992). The association of the Met235Thr polymorphism and blood pressure response to antihypertensive is confused. It seems that the Met235Thr variant may be important as a predictor of patient response to ACE inhibitor therapy (Hingorani et al., 1995; Kurland et al., 2004), illustrating the potential use of SNP genotyping as a pharmacogenetic tool in antihypertensive treatment. However, a meta-analysis of more than 45 000 subjects (Sethi et al., 2003) revealed that genotype did not predict plasma angiotensinogen levels in Asian and black subjects, hypertension in black subjects, or systolic or diastolic blood pressure in either ethnic group. Measurements of AGT levels and genotyping of *AGT* polymorphisms is highly dependent on ethnicity.

In contrast, previous works reported that increases in high-molecular weight angiotensinogen (glycosylated), but not low molecular-weight angiotensinogen (nonglycosylated) were associated with pregnancy-induced hypertension in woman (Tewksbury and Dart, 1982). Moreover, glycosyled AGT was a better substrate for renin, compared with low-molecular weight angiotensinogen (Tewksbury and Tryon, 1989). Consequently, post-traductional modifications of angiotensinogen could complete informative measurements of AGT. In

conclusion, interest of individual genotyping for *AGT* polymorphisms to predict blood pressure response is clear; however, which *AGT* polymorphism could be taken into consideration is unclear, particularly since the association of *angiotensinogen* gene haplotypes with hypertension is discussed (Brand-Herrmann et al., 2004; Renner et al., 2005).

Fortunately, other genes are predictive of blood pressure (BP)-response to drug and prediction of the effect of medication on BP is highly dependent of the drug taken into consideration. Thus, the C825T polymorphism of the *G-protein β3* gene appears to predict patient response to thiazides diuretics (Turner et al., 2001), while CA repeat length of the *11-β-hydroxysteroid dehydrogenase type 2* (11β HSD2) gene was strongly associated with the BP response to hydrochlorothiazide (Williams et al., 2005). The *11β HSD2* G534A polymorphism can cause a rare form of salt-sensitive monogenic hypertension (Lifton, 1996) and is proposed as "salt-sensitive" marker (Poch et al., 2001). Interestingly, a microsatellite CA repeat marker in intron 1 of the *HSD11B2* gene was associated with the urinary cortisol metabolites ratio reflecting a mild reduction in HSD11B2 activity, which in turn was significantly related to plasma renin activity levels. Although the CA repeat polymorphism was not associated with BP levels, ratio has been demonstrated to be the best indicator of HSD11B2 activity in vivo, and displays a lower intra-individual variability and a better discrimination between salt-sensitive and salt-resistant subjects compared to the ratio of the urinary free glucocorticoids (Ferrari et al., 2001). In this occurrence, metabolite ratio could be more predictive of salt-sensitive hypertension than *HSDB2* polymorphism genotyping.

4.2. Aldosterone-to-renin ratio

The prevalence of primary aldosteronism is less than 2% within the hypertensive population and is characterized by an excess production of the normal adrenal hormone, aldosterone, low serum potassium and also a suppressed plasma renin. Tests, looking at other adrenal steroid hormones, can be very useful as well as tests looking for the normal physiologic changes in hormones in the morning and evening, as well as responses to sodium challenge or sodium restriction. Body sodium has been established to change with age in hypertensive patients and correlated with blood pressure (Beretta-Piccoli et al., 1982). In the same manner, aldosterone–renin ratio was positively related to age and plasma sodium concentration in hypertensives (Komiya et al., 1997), relationships that could not be detectable using plasma aldosterone levels (Reubi and Weidmann, 1980) and with blood pressure in hypertensives (Lim et al., 2001). As suggested by Lim et al. (2002), aldosterone–renin ratio might help to isolate patients with inappropriate aldosterone activity who would respond favourably to aldosterone antagonists. However, although several studies have shown the favourable blood pressure-lowering effects of aldosterone antagonists (Lim et al., 2002; Brown, 2001), these results need to be investigated in large cohorts. As previously suggested, aldosterone–renin ratio seems to have a greater predictive power in blood pressure response to diuretics than aldosterone levels. This could also explain why the aldosterone C-344T polymorphism that could influence aldosterone levels is not found to be associated with a greater blood pressure reduction in response to diuretics (Liljedahl et al., 2003; Frazier et al., 2004).

4.3. Inflammation and hypertension

The overall contribution of inflammation to vascular damage in patients with hypertension is still unclear even after several investigations (Clozel et al., 1991; Dzielak, 1992). Inflammation has been associated with decreased endothelium-dependent relaxation, a process related to an alteration in the bioavailability of nitric oxide, and there is considerable evidence of a link between endothelial dysfunction and both essential and pregnancy-induced hypertension (Teran et al., 2000). The most recent and most promising biochemical marker is with no doubt high soluble C-reactive protein (CRP) that appears as a strong predictor of future cardiovascular disease (Rifai, 2005). A previous investigation had reported that there was a genetic contribution to baseline serum concentrations of C-reactive protein (MacGregor et al., 2004). A higher prevalence of hypertension related to higher levels of C-reactive protein (Schillaci et al., 2003) was also described. Therefore, greater plasma C-reactive protein concentrations were found in patients with hypertension than in normotensive healthy controls (Bautista et al., 2001) and were directly associated with systolic blood pressure and pulse pressure in newly diagnosed, never-treated hypertensive patients (Bautista et al., 2001). Moreover, a positive relationship between increased serum levels of C-reactive protein and the risk for development of incident hypertension in participants of the Women's Health Study (Sesso et al., 2003) was also described. Finally, high levels of C-reactive protein may upregulate angiotensin receptors and enhance expression of plasminogen activator inhibitor-1 by endothelial cells (Grundy, 2003). C-reactive protein levels measurements reflect inflammation that could lead to hypertension. Both investigations need to take genetic variants and soluble level measurements into consideration. It seems clearly of interest to also consider metabolite as a putative marker of the disease and adverse effects.

5. Inflammation, a general physiopathological involvement

As mentioned beyond, it seems that inflammation is an important component of hypertension. It is also a physiopathological state and a sub-clinical cause of other risk factors leading to cardiovascular diseases (e.g. metabolic syndrome, diabetes, obesity). Thus, pharmacogenomics of cardiovascular drugs should take into account this metabolic deviation.

More than 100 genes are related to inflammation if we take into account those regulated by nuclear factor-κB (NF-κB), Activator protein-1 (AP-1), peroxisomal proliferation-activating receptor-α (PPAR-α), etc. Inflammation biomarkers, which are measurable in blood, could be divided in five groups: acute phase reactants (e.g. C-reactive protein, serum amyloid A protein, and fibrinogen), cytokines (e.g. interleukin-1 beta (IL-1β),

interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α)), chemokines (e.g. monocyte chemoattractant protein-1 (MCP-1)), adhesion molecules (soluble forms) and proteases; in addition to lipoprotein-associated phospholipase A2 (Lp-PLA_2) and CD40 ligand (McConnell, 2005).

Many polymorphisms could influence their level in circulation (Berrahmoune et al., 2005). Some of them have been studied in relation to cardiovascular risk. Indeed, they could affect balance of cytokine network that can lead in addition to some environmental risk factors, to disrupt cytokine equilibrium and improve atherogenesis (Alam et al., 2004). Finally, they could also have an impact on cardiovascular therapy (Table 5).

One of these polymorphisms, 915G/C of *TGF-β1* gene was found to be associated with molecule level and anti-hypertensive treatment effect (Hallberg et al., 2004). Indeed, these drugs are known for a long time to have an anti-inflammatory effect, like angiotensin-converting enzyme inhibitors (Martin et al., 1984). Considerable evidence now supports a role for angiotensin II as a pro-inflammatory mediator, elevating it to the category of an "honorary" cytokine. It can for example elicit vascular cell adhesion molecule 1 and MCP-1 expression by endothelial cells, and IL-6 production by smooth muscle cells (Libby, 2002).

Irbesartan-treated patients who were carriers of the C-allele at position 915, which is associated with low expression of TGF-β1, responded with a markedly greater decrease in left ventricular mass index than subjects with the G/G genotype, independent of blood pressure reduction (Hallberg et al., 2004).

Another polymorphism in *IL-1β* gene (−511) seems to correlates in vitro with the production of IL-1β by mononuclear cells (Pociot et al., 1992). A more recent study showed that this production increased a slight but non-significantly in individuals with a T allele of this SNP. However, an IL-1 receptor antagonist gene, with which the −511 SNP was in linkage disequilibrium, was a stronger determinant of raised IL-1β production (Santtila et al., 1998). Nevertheless, Kornman et al. found that *IL-1β* (−511) polymorphism was important in combination with *IL-1RN* (+2018) marker and was associated with atherosclerotic plaque formation, as measured by angiography and arterial wall thickness (Kornman et al., 1999). In addition, to be involved in atherogenesis, *IL-1β* (−511) polymorphism has been shown to interact with a cardiovascular drug: pravastatin, which exerts direct anti-inflammatory effects like other statins (as cited beyond). In addition, studies on Cholesterol and Recurrent Events (CARE) trial and the Air Force/Texas Coronary Atherosclerosis Prevention Study (AFCAPS/TexCAPS) suggest that statin therapy may be differentially effective among those with inflammation compared with those without (Ridker et al., 1998; Ridker et al., 2001).

Another study on effect of *IL-1β* −511 C/T polymorphism on treatment by pravastatin indicates that, after 6 months of treatment, in men with *IL-1β* C allele, levels of IL-1β decreased, while in men with the T allele, it increased; however, this difference is not very significant ($p=0.061$). In addition, researchers found that changes in adenosine-stimulated flow and coronary flow reserve with pravastatin were significantly dependent on *IL-1β* genotype. They explained their results by the fact that statin treatment decreased IL-1β level more effectively in subjects with CC genotype, thus leading to lower endothelial inflammatory response, and better endothelial function as indicated by increased adenosine-stimulated flow and coronary flow reserve (Lehtimaki et al., 2003).

IL6-174G/C genotype is a much studied one as it is correlated with IL-6 level in circulation (Haddy et al., 2005). It is related to carotid IMT (Rundek et al., 2002), peripheral artery

Table 5
Polymorphisms influencing protein concentration, cardiovascular disease and treatment

Gene	SNP	Effect of polymorphism on			Effect of protein on treatment	References
		Protein concentration	Cardiovascular disease	Treatment		
IL-1β	−511 C/T	−511T/T → ↑ IL-1β level	Atherosclerotic plaque formation (in combination with IL-1RN (+2018) marker)	−511C/C→ ↑ Effect of pravastatin		Pociot et al., 1992; Kornman et al., 1999; Lehtimaki et al., 2003
IL-6	−174G/C	−174C/C → ↑ IL-6 level −174G/G → ↑ IL-6 level	Carotid IMT, peripheral artery occlusive disease and retinal artery occlusion	−174C/C → ↑ Effect of pravastatin −174 G/G → Effect of enalapril and losartan		Haddy et al., 2005 Rundek et al., 2002; Flex et al., 2002; Weger et al., 2005; Burzotta et al., 2001; Trevelyan et al., 2004
CRP	−286 C/T/A	−286 CA/TA → ↑ CRP level[a]			↑ CRP level → ↑ effect of aspirin	Kovacs et al., 2005
	1059G/C	1059G/G→ ↑ CRP level				Suk et al., 2005
	1444C/T	1444T/T→ ↑ CRP level				Brull et al., 2003
TGF-β1	915G/C	915G/G→ ↑ TGF-β1 level		↓ LVM index by irbesartan is more efficient for CC genotypes		Hallberg et al., 2004

IL-1β: interleukin-1 beta; IL-1RN: interleukin-1 receptor antagonist; IL-6: interleukin-6; CRP: C-reactive protein; TGF 1: transforming growth factor beta 1; IMT: intima media thickness, LVM: left ventricular mass.

[a] In patients with myocardial infarction and not in healthy individuals.

occlusive disease (Flex et al., 2002) and retinal artery occlusion (Weger et al., 2005).

IL-6 polymorphism also interacts with pravastatin as men treated with this drug for one year carrying GG+GC genotype, had 25% lower risk (OR=1 reduced to OR=0.75), compared with the GG+GC placebo group; whereas in the CC group, risk was 77% lower than that in the CC placebo men (OR reduced from 1.19 to 0.42). In addition, the reduction in LDL cholesterol was greater in the CC group than in the GG+GC group. Larger declines in fibrinogen and C-reactive protein and a larger elevation in HDL levels in the CC group were found compared with the GG+GC group; these latter differences were, however, statistically not significant. Researchers hypothesized that the protective effect of the CC genotype in the treated group was likely to be due to either a greater pravastatin lipid-lowering effect or a greater inflammation-lowering effect in this genotype group. The analysis of the WOSCOPS data suggests that both of these effects may be involved (Basso et al., 2002).

In addition, in randomized men awaiting coronary artery bypass graft, enalapril produced a highly significant decrease of 51% in the release of IL-6 in patients identified as high producers of IL-6 by the −174 G/C polymorphism, whereas losartan has a similar but less marked effect (Trevelyan et al., 2004).

On the other hand, results from the Physicians Heart Study showed that the benefit of aspirin treatment was greatest in subjects with elevated baseline C-reactive protein levels (Ridker et al., 1997). This finding is confirmed by other results indicating that the effect of aspirin in preventing a first myocardial infarction was greatest among men with the highest baseline C-reactive protein concentrations and that the benefit diminished significantly with decreasing concentrations of this inflammatory marker (Vane, 1987).

These results suggest that the benefit of anti-inflammatory treatment may be greatest in those with highest inflammation and so in part, in individuals who bring alleles of polymorphisms that contribute to increase gene expression e.g. *IL-1β* (−31C/T (Hamajima et al., 2001), *IL-6* (−572G/C (Haddy et al., 2005)), *CRP* (−286 C/T/A (Kovacs et al., 2005), 1059G/C and intronic T/A (Suk et al., 2005) and 1444C/T (Brull et al., 2003)).

Moreover, proteomics could be very helpful in order to follow drug effect on inflammation process. Indeed, for example, treatment with enalapril and losartan decreases releases of IL-1 receptor and IL-6 in patients undergoing coronary artery bypass graft with cardio-pulmonary bypass (Trevelyan et al., 2004).

Other drugs are known to have an anti-inflammatory effect like lipid lowering drugs which stabilize atherosclerotic plaques by decreasing inflammation (Weissberg, 1999). Fibrates are activators of PPARα, which stimulates β-oxidation of fatty acids. It was reported that in addition to their role, fibrate have anti-inflammatory action, at least in part, by negatively regulating NF-κB transcriptional activity. They inhibit interleukin-1-induced production of interleukins-6 and prostaglandin and expression of cyclooxygenase-2 (Staels et al., 1998).

It was shown that fenofibrate significantly decreases soluble intercellular adhesion molecule 1 (sICAM-1) (by 17%) and MCP-1 levels (by 12.5%) when simvastatin causes a significant decrease (by 10.5%) in sICAM-I only (Kowalski et al., 2003).

Whereas inflammation plays a decisive role in atherogenesis, anti-inflammatory drugs could not be used in prevention or treatment of cardiovascular affections. In fact, these drugs interact with many others drugs, which are used in patients with cardio- or cerebrovascular disorders: They attenuate the effects of diuretics, β-blockers, ACE inhibitors and angiotensin II receptor blockers and potentiate the effect of oral anticoagulants and interact with platelet inhibitors (Stollberger and Finsterer, 2003).

Personalized medicine is based on differences between individuals and aspires to give for each one adequate treatment according to polymorphisms he brings. However, it could be interesting in addition to study these polymorphisms, to take into account proteins to follow treatment effect. It is so important to be able to measure new molecules instead of those routinely quantified (e.g. C-reactive protein, orosomucoid, haptoglobin, serum amyloid A, albumin, fibrinogen and plasminogen activator inhibitor-1 (PAI-1)) like cytokines (e.g. IL-1β and IL-6). The most common technique used to measure cytokine level is ELISA. However, this method is not yet randomised in clinical laboratories and there is a need to a rigorously standardized and reproducible assay. Indeed, many factors influence the outcomes of these assays, according to manufactures used, collection and storage procedures (Aziz et al., 1998).

In addition, biological and environmental factors contribute to interindividual variation in cytokine level like body mass index (Haddy et al., 2005). Indeed, various inflammatory cytokines (leptin, TNF-α, IL-6, adiponectin) and thrombotic molecules (PAI-1) cytokines are synthesized by adipocytes (You et al., 2005). In addition, obesity is a state of chronic inflammation, as indicated by increased plasma concentrations of C-reactive protein, IL-6, and PAI-1 in obese subjects. Novel data have now appeared showing that the concomitant presence of promoter polymorphisms of *TNF-α* (−308G/A) and *IL-6* (−174C/G) in these subjects with impaired glucose tolerance carry twice the risk of conversion to type 2 diabetes when compared with other genotypes. A −308G/A mutation of the *TNF-α* promoter is associated with increased plasma TNF-α concentrations and a 1.8 higher risk of developing diabetes compared to non-carriers. A −174C/G mutation of the *IL-6* promoter increases the risk for insulin resistance (Dandona et al., 2004). That is why anti-diabetic drugs also have anti-inflammatory effects. For example, thizolidinediones decrease C-reactive protein, fibrinogen and PAI-1 levels, inhibit NF-κB and decrease TNF-α effect (Zarich, 2003). Cytokines and adipocytokines could be so good biomarkers to evaluate anti-diabetic drug effects.

6. Diet, body mass index and obesity. Example of gene–environment effects in cardiovascular events

Many genes are reacting differently to physiological variables such as age, gender and also their polymorphisms are

different for ethnicity due to human evolution. All the previous description and recommendations have to take these variations into account before looking to a pharmacogenomic effect of a cardiovascular drug. That is particularly well known for drug metabolizing enzymes genes. Furthermore, referring to the environment, tobacco smoking, alcohol and drug consumption, diet and body mass index are crucially implicated in the development and progression of cardiovascular disease.

Dietary recommendations are the first therapeutic approach in cardiovascular diseases. A diet low in total fats (particularly these of animal origin), low in cholesterol, and high in antioxidant elements has showed favorable outcomes in patients and an improved quality of life in healthy subjects. The response to diet and the interactions between diet and disease depend on genetic polymorphisms (nutrigenetics). In the case of cardiovascular disease the most established gene–nutrients interactions concern the dietary fats and genes involved in lipid metabolism (apolipoproteins, lipoprotein lipase and hepatic lipase among others). The apolipoprotein E gene remains the locus most consistently reported with respect to gene environment. In subjects carrying the apoε4 allele, a low-fat, and low-cholesterol strategy may be particularly beneficial in terms of lowering plasma cholesterol levels (Siest et al., 2000; Ordovas and Mooser, 2004). The same strategy can be possibly applied in altering the HDL concentrations, after dietary intake of polyunsaturated fatty acids (PUFA). It has been reported recently that their effect on HDL-cholesterol concentrations is modulated by a common genetic polymorphism in the promoter region of the *APOA1* gene. Thus, subjects carrying the A allele at the −75G/A polymorphism show an increase in HDL-cholesterol concentrations with increased intakes of PUFA, whereas those homozygotes for the more common G allele have the expected lowering of HDL-cholesterol levels as the intake of PUFA increases. Subsequently, it could be predicted that subjects with low levels of HDL-cholesterol and carriers of the A allele may benefit from diets containing higher percentages of PUFA. A third very interesting example has been recently reported focusing on the interaction between intake of animal origin fat and variability at the hepatic lipase gene, encoding a key enzyme involved in reverse cholesterol transport. These has been shown, that subjects carrying the CC genotype (the most common among Caucasian subjects) "react" to high contents of fat in their diets by increasing the concentrations of HDL-cholesterol, which could be interpreted as a "defense mechanism" to maintain the homeostasis of lipoprotein metabolism. Conversely, carriers of the TT genotype experience decreases in HDL-cholesterol levels (Ordovas and Mooser, 2004). Beyond citing these interesting examples, it is important to note that there have been studies extensively on other genetic variants concerning mostly the APOAI, the APOAIV, the APOCIII and the APOB; some examples of which are cited in the Table 6 (Masson et al., 2003).

In the case of obesity, there has been a rapid increase the last years in the understanding of biochemical events thought to be causative factors aiming thus in the development of new therapeutic approaches. The new generation of drugs targeting obesity focalize in (i) reducing energy intake (leptin and leptin receptors, pro-opiomelanocortin and melanocortin receptors, neuropeptide Y and its receptors, endocannabinoids and cannabinoid CB1 receptor, etc.), (ii) increasing energy expenditure (uncoupling protein 1, β_3-adrenoceptors, PPAR-γ modulators) and (iii) producing thermogenic effects (β_3-adrenoceptors, PPAR-δ agonists) (Jandacek and Woods, 2004; Nisoli and Carruba, 2004). In clinical practice, the current drugs for long-term treatment of obesity are sibutramine and orlistat which are designed to reduce food intake and utilization of ingested energy respectively. Sibutramine is a serotonin, norepinephrine and dopamine reuptake inhibitor, while orlistat inactivates pancreatic lipase thereby inhibiting hydrolysis and absorption of dietary triacylglycerol. The approach of orlistat

Table 6
Examples of apolipoprotein polymorphisms modifying diet response

Polymorphism and study	Subjects (*n*)	Genotype groups (*n*)	Intervention	Response			
				Cholesterol	LDL	HDL	TG
Apolipoprotein AI −75 (G/A)							
Lopez-Miranda et al.	89 males	G/G, 58 G/G, 31	NCEP-I vs. high fat, high MUFA diet	S		NS	NS
Apolipoprotein CIII C1100T							
Humphries et al.	55 males and women	C/C, 38 C/T and T/T, 17	High SFA vs. high PUFA diet	NS	NS	NS	NS
Apolipoprotein AIV Thr347Ser							
Jansen et al.	41 males	Thr/Thr, 25 Ser (Thr/Ser and Ser/Ser), 16	NCEP-I vs. high MUFA diet	S	NS	NS	NS
Apolipoprotein B Asn1887Ser							
Ilmonen et al.	54 females	Asn/Asn, 52 Asn/Ser, 2	Low fat, low cholesterol, high P/S diet	NS	NS		

Adapted from Masson et al. (2003).
HDL: high-density lipoprotein; LDL: low-density lipoprotein; MUFA: monounsaturated fatty acids; NCEP: National Cholesterol Education Program; P/S: polyunsaturated to saturated fatty acid ratio; PUFA: polyunsaturated fatty acids; SFA: saturated fatty acids; TG: triglycerides.
NS: non-significant; S: significant.

has successfully achieved modest long-term reductions in body weight. Recently, the effect of weight reduction with orlistat treatment on lipid peroxidation levels, which is found to be associated with obesity, was investigated.

In general, the outcomes of the existing drugs targeting obesity are evaluated in clinical practice by markers as the weight loss and the waist circumference. The identification and determination of biomarkers independently related to body mass index appears to be the most promising approach for better evaluation of drug effects. The secretion of molecules from the adipose tissue, which are characterized as "adipocytokines", is a new interesting field towards this approach. More precisely, leptin and adiponectin are hormones produced mainly by the adipose tissue and the blood concentration of these molecules depends on the body mass index (see beyond). They are mainly implicated in the energy metabolism and recently there have been developed several assays to measure them (Meier and Gressner, 2004). In the case of leptin, the serum levels of this hormone are considered as an indicator to predict the clinical efficacy of troglitazone (antidiabetic drug) in patients with type 2 diabetes by measuring its levels before treatment (Maruyama et al., 2001). As adipocytokines are implicated in glucose regulation and lipid metabolism, the association of their plasma concentrations with markers of triglyceride-rich lipoprotein metabolism was studied. Interestingly, adiponectin was an independent predictor of plasma apoB-48, apoC-III, RLP-cholesterol and triglycerides, where leptin was not, suggesting that plasma adiponectin levels may not only link abdominal fat, insulin resistance and dyslipidemia, but may also exert an independent role in regulating triglyceride-rich lipoprotein metabolism (Chan et al., 2005). Furthermore, saturated and ω-3 fatty acids plasma concentrations were significantly correlated with plasma adiponectin levels, showing a negative and a positive correlation respectively (Fernandez-Real et al., 2005). Many other studies demonstrate the utility of circulating adiponectin as a biomarker of the metabolic syndrome. This relationship includes decreased adiponectin levels in increased body mass index, decreased insulin sensitivity, less favourable plasma lipid profiles, increased levels of inflammatory markers and increased risk for the development of cardiovascular disease. Therefore, adiponectin levels hold great promise for use in clinical application serving as a potent indicator of underlying metabolic complications (Trujillo and Scherer, 2005). Besides its possible role as biomarker, adiponectin can be also considered as drug target in improving hepatic insulin sensitivity. Thiazolidinedione antidiabetic effects in the liver may be mediated through the upregulation and increase secretion of adiponectin from adipocytes after stimulation of PPARγ in adipocytes (Bouskila et al., 2005).

The fact that many genes are implicated in obesity gives rise to new drug targets but also verifies the complexity of this phenomenon and underlies the importance of considering the genetic variants of these genes for evaluating the outcomes of drug therapeutic interventions. To date, there exist 358 findings of positive associations between obesity phenotypes and genetic variations of 113 candidate genes (Perusse et al., 2005).

Genetic variants of leptin and its receptor are related to obesity and increased body mass index, while polymorphisms in the gene of adiponectin result in phenotypes such as insulin resistance, type 2 diabetes additionally to obesity and increased adiposity. Interestingly, variations in both genes can result in altered circulating levels of these two proteins (Cancello et al., 2004), signalling the importance of taking these polymorphisms into account when tending to use these molecules as biomarkers. To mention, there are cited polymorphisms of dopamine receptors (DRD2, DRD4) related to obesity and of hepatic lipase related to abdominal visceral fat and to body mass index (Perusse et al., 2005), proposing possible variations in drug response in the case of sibutramine and orlistat.

7. Pharmacoproteomics as a future

Through the different examples we have taken, numerous enzymes, proteins, peptides, receptors can be used as phenotypes to enter in the pharmacoproteomic follow-up of cardiovascular drugs.

In Fig. 3, we put some examples of proteomic markers currently measured in laboratory medicine. However, pharmacoproteomics of cardiovascular drugs is more than that. We should follow the same large scale strategy as for SNP genotyping and expression profiling using the powerful methods of 2-D gel electrophoresis (Anderson and Anderson, 1996), mass spectrometry (Fathi et al., 2003) and protein chips (Wilson and Nock, 2002).

In a recent presentation on pharmacoproteomics in the Santorini Conference, Langen (2004) ("Proteomics Applications in Pharmaceutical Industry", Santorini October 2004) presented an industrial strategy for searching biomarkers linked to diabetes and useful in drug development. He was proposing to start with cellular models, pancreatic beta cells which loose their insulin secreting capabilities after a history of disturbed blood glucose uptake regulation and insulinoma cells. Through many proteomics spectra done in this cell line he could find 4.000 proteomics spots for approaching the different metabolic pathways. After this huge number of mass spec and quantification of the proteins, he was able to describe several proteins and enzymes which could be proposed as proteomics biomarkers for this disease.

For statins, a comparable strategy has been used for following the protein modification in rat liver after administration of lovastatin (Steiner et al., 2000) and fluvastatin (Steiner et al., 2001). In these studies, the group of N.L. Anderson, with his experience on 2D gels and using also MS/MS for specific confirmation, has described key modification in carbohydrate metabolism, stress protein, calcium homeostasis and protease activity. It has been suggested to follow other enzymes from the mevalonate pathway, an important information which could have avoid the problem with cerivastatin or some other side effects.

Proteomics approaches are clearly very useful during the development of new drugs (Witzmann and Grant, 2003) to control some toxicities including drug interactions and in different pathological states (Aldred et al., 2004). However, for

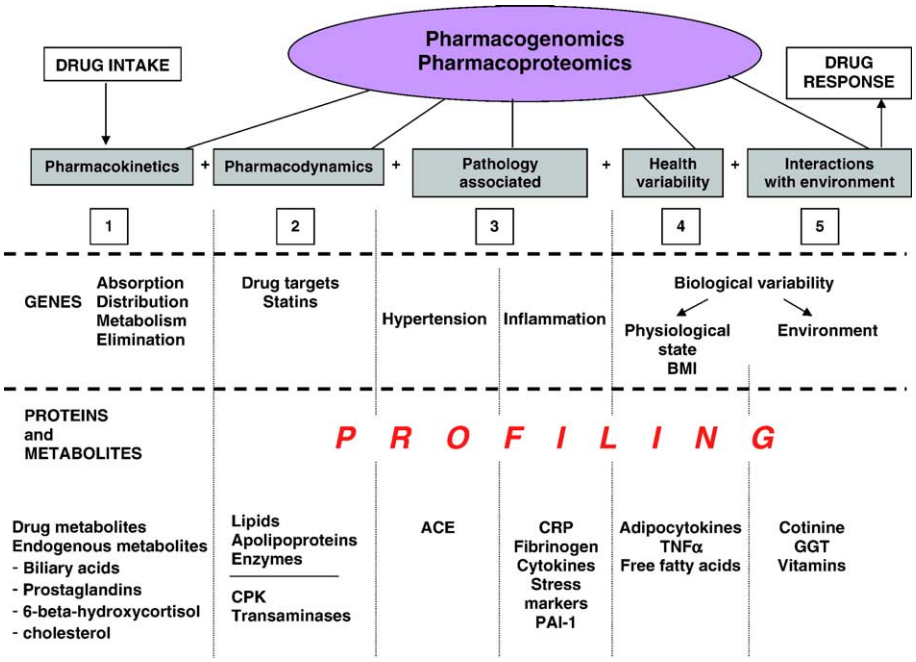

Fig. 3. Examples of pharmacoproteomic markers measured in addition to each of the five groups of pharmacogenomic markers. ACE: angiotensin-converting enzyme; BMI: body mass index; CPK: creatine phosphokinase; CRP: C-reactive protein; GGT: gamma glutamyl transferase; TNFα: tumor necrosis factor alpha.

doing a more specific use of pharmacoproteomic in the cardiovascular field, we need to better know the proteome profile of the organs involved: heart (Jungblut et al., 1994, 1999), vessels, and specific blood cells including lymphocytes. Here again looking for a set of specific protein, related if possible to a metabolic pathway, i.e. stress protein, will be very useful (Scheler et al., 1997). The relation with other pathologies can bring also interesting information i.e. proteomic information on inflammation markers in rheumatoid arthritis (Mattila and Frey, 1995) (Doherty et al., 1998) or oxidation in Alzheimer patients. Finally, proteomic approach could be applied to dissect the composition of particles which are involved in risk assessment i.e. LDL or HDL which are heterogeneous. Profiling such particles will probably be a real progress in knowing the composition related to the functional role of their constituents (Karpe and Hamsten, 1994).

Cardiovascular diseases cannot be studied as themselves, the clinical expression of this multifactorial diseases being too heterogeneous (Bougneres, 2003). We need focus on metabolic pathways linked to cardiovascular disorders and which are the real targets of the drugs and their individual pharmacogenomic and pharmacoproteomic responses.

We should take into account, after analysis of the literature (Persidis et al., 2004), the systems "biology approach" proposed, i.e. by Kitano (2002). System-level understanding, the approach advocated in systems biology (Van Ommen, 2004), requires a shift in our notion of "what to look for" in biology. While an understanding of genes and proteins continues to be important, the focus is on understanding a system's structure and dynamics. Because of system is not just an assembly of genes and proteins, its properties cannot be fully understood merely by drawing diagrams of their interconnections. Although such a diagram represents an important first step, it is analogous to a static roadmap, whereas what we really seek to know are the traffic patterns, why such traffic patterns emerge, and how we can control them.

In the same spirit Leroy Hood and his team (Ideker et al., 2001) has used such an integrated approach in studying through different presentations, the yeast galactose pathway and the response of 1000 messengers RNAs. Using the same yeast model with 6000 protein arrays, Hall et al. (2004) were able to describe new protein/DNA interactions important in controlling metabolic pathways. Amoeba has also been used as a model cell with 10 000 genes homologues or equivalents of genes found in humans (Van Driessche et al., 2005). It will soon be necessary to apply such strategies to higher eukaryotic cells and to test with these models new cardiovascular drugs and to discover new markers.

Genomic and proteomic biomarkers are biological measurements that serve as indices for disease progression, pharmacology or safety — they have become increasingly useful as a basis for decision-making in drug development. Used independently as indicators or in combination to produce a pattern profile, biomarkers can reflect a variety of disease characteristics, including the level of exposure to an environmental or genetic trigger.

With their potential use as validated surrogate endpoints to indicate drug efficacy or toxicity, biomarkers are emerging as the solution to many of the problems currently facing pharmaceutical companies.

Genomic and proteomics markers for cardiovascular drugs should be used to pinpoint individuals at high risk for the disease and at risk for the drug side effects or with a probability of nonresponse.

The practice of personalized medicine should use the huge amount of genomic, proteomics and metabolomic data which

will be developed during the next years with the powerful arrays technologies (Ruano et al, 2004). However, we should not forget that Humans are living in special environments regulating the expression of genes and genes products. Nutrition, tobacco, alcohol, obesity, and other drugs, such as contraceptives, have been particularly linked to the pharmacogenomic and pharmacoproteomic strategy for cardiovascular drugs.

In conclusion, for such multifactorial chronic diseases as cardiovascular ones, we have to dissect them into separate metabolic entities for selecting genes and gene products involved in each pathway. Following our five-step strategy, a cardiovascular drug could be adapted to each patient or to a subgroup of patients if each of this step can be followed with one to 10 genes and one to 10 proteomic or metabolic markers. We will then have to propose to clinicians a pharmacodiagnosis profile with an adapted interpretation using a multidimensional profile.

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