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Guiding Hypertension Treatment with Pharmacogenomics:

The Role of the Community Pharmacist

Dissertação de Mestrado em Ciências Farmacêuticas, especialidade Biotecnologia Farmacêutica, orientada pelo Professor Doutor Sérgio Simões e apresentada na Faculdade de Farmácia da Universidade de Coimbra

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ABSTRACT

Hypertension is a major risk factor for heart disease and stroke, which are the leading causes of death worldwide. Anti-hypertensive drugs account for the major drug class used in the treatment and prevention of cardiovascular diseases, although their use is based on a trial-and-error rationale. For anti-hypertensive drugs the percentage of non-responders can reach 20% of the patients under treatment, and non-compliance due to adverse drug responses is frequent.

Among the causes known which account for differences in drug response are the interindividual genetic differences - the focus of study in Pharmacogenomics. Being able to provide Pharmacogenomic tests in a community pharmacy setting gives the pharmacist an opportunity to individualize drug therapy, maximising efficacy and minimising toxicity, based on patients' genetic data.

Given the number of hypertensive patients (*circa* 30% of global population), the expenditure with cardiovascular drugs (one million euros per day in reimbursements in Portugal) and the difficulty in finding the right drug for each patient, being able to offer Pharmacogenomic tests to hypertensive patients in a community pharmacy setting is an opportunity that should be seized.

Keywords: Hypertension, Pharmacogenomics, Community Pharmacy.

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Acronyms

ACE - Angiotensin-Converting-Enzyme

ACEI - Angiotensin-Converting Enzyme Inhibitors

ADME - Absorption, Distribution, Metabolism, Excretion

ADRs - Adverse Drug Responses

Ang - Angiotensin

ARB - Angiotensin II Receptor Blockers

AT - Angiotensin Receptor

CV - Cardiovascular

CVD - Cardiovascular Diseases

CYP - Cytochrome P450

DBP - Diastolic Blood Pressure

DNA - Deoxyribonucleic Acid

EMA - European Medicines Agency

ESC - European Society of Cardiology

ESH - European Society of Hypertension

FDA - Food and Drug Administration

HCTZ - Hydrochlorothiazide

HT - Hypertension

mRNA - Messenger Ribonucleic Acid

NGS - Next-generation sequencing

PCR - Polymerase Chain Reaction

PD - Pharmacodynamics

PGx - Pharmacogenomics

PK - Pharmacokinetics

PM - Personalized Medicine

R&D - Research & Development

RAAS - Renin-Angiotensin-Aldosterone System

SBP - Systolic Blood Pressure

SNPs - Single Nucleotide Polymorphisms

WHO - World Health Organization

1. Introduction

Hypertension, or high blood pressure, is a major risk factor for heart disease and stroke, which are the leading causes of death worldwide [1]. According to the World Health Organization (WHO), as depicted in Figure 1, it is only in the low income countries that heart disease does not account for the major cause of death.

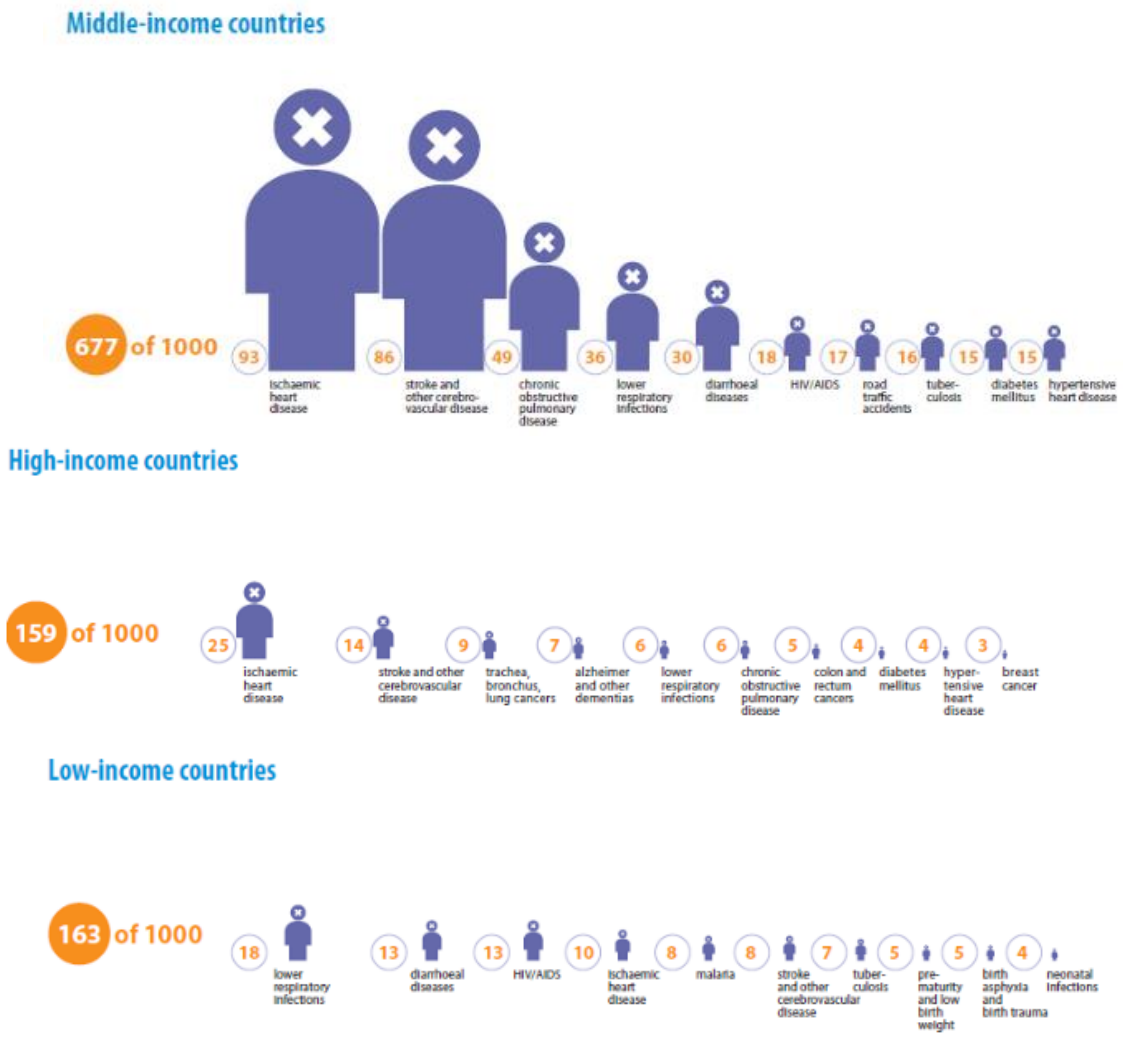

Figure 1 - Top ten causes of death across the world

(Adapted from the World Health Organization Fact Sheets, accessed in September 2012)

In a scientific study published in 2005[2], it was shown that in the year 2000, 26.4% of the adult population had hypertension (26.6% of men and 26.1% of women), and 29.2% were projected to have this condition by 2025. The estimated total number of adults with

hypertension in 2000 was 972 million; 333 million in economically developed countries and 639 million in economically developing countries. The number of adults with hypertension in 2025 was predicted to increase by about 60% to a total of 1.56 billion.

In the *World Health Statistics 2012*[3] report published by the WHO, it was reported that in 2008, in Portugal, under the known causes for mortality between 30 and 70 years old per 100.000 inhabitants, the deaths caused by cardiovascular diseases (CVD) and diabetes accounted for 23% of all causes of death. In the European region this number rises up to 38% of all causes of death. World-wide the percentage of deaths caused by CVD and diabetes is 32%.

According to the same report, the prevalence of raised blood pressure among adults aged above 25 years old, in 2008, in Portugal, was of 34,5% and 24,3% of the male and female population, respectively. Globally, these number accounted for 29,2% of the male population and 24,8% of the female population.

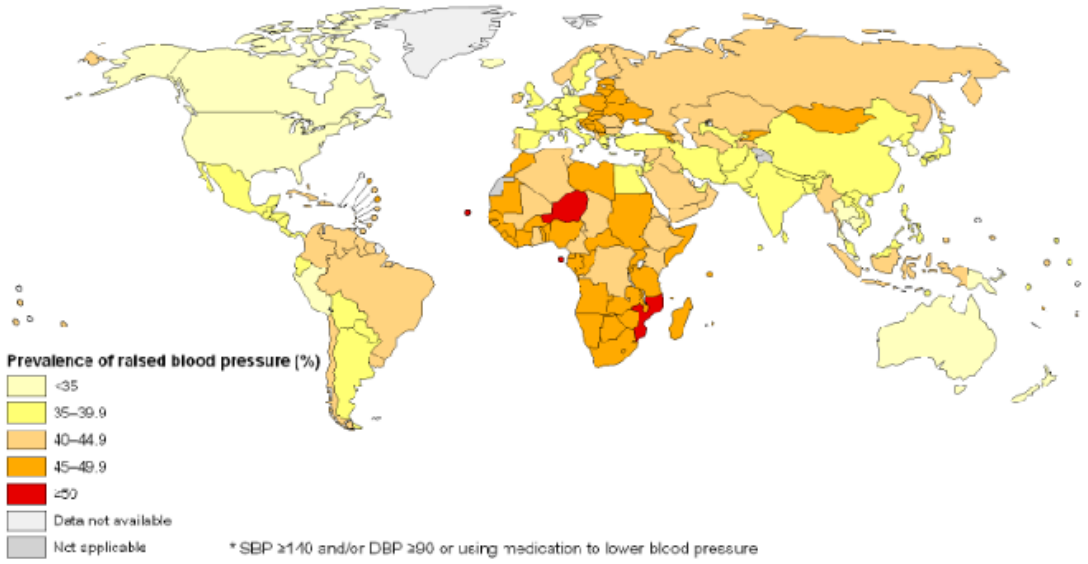

Figure 2- Prevalence of raised blood pressure among adults age 25+, both sexes, 2008. (Adapted from the World Health Organization – Map Gallery)

What was predicted, in 2005, to happen in 2025 (29,2% of hypertensive population) has been reached in a far shorter time.

Awareness, prevention, treatment and control of hypertension are significant public health measures and cost-effective interventions. Nevertheless, and despite the existing theoretically knowledge necessary to prevent and control hypertension, control rates are dim in every part of the world.

Many hypotheses have been laid to respond to this fact, one of which is the vast array of risk factors for hypertension that include dietary habits, high alcohol consumption, low levels of physical activity and overweight. As these are modifiable risk factors, the primary treatment includes lifestyle changes. When lifestyle modifications fail to control hypertension, pharmacotherapy is essential. Nevertheless, 15% to 20% of all patients fail to respond to drug treatment, even with a two-drug combination [(4)].

In addition, the responses to anti-hypertensive drugs differ significantly between patients, as they depend on several factors such as gender, lifestyle, age, race, disease severity, disease progression, underlying illnesses, concomitant pharmacotherapy, patient's compliance and individual genetic makeup.

Optimal blood pressure control requires increased understanding of the evaluation and management of blood pressure, and it is important to be aware that the individual genetic variability plays an important role in the differences in drug response.

These individual genetic differences - deoxyribonucleic acid (DNA) polymorphisms - are studied under the emerging science of **Pharmacogenomics** (PGx), which has as its ultimate goal to identify the underlying genetic factors that play a role in the efficacy and toxicity of all drugs[(5)].

Moreover, PGx research refers to the use of appropriate DNA methodologies to develop reliable biomarkers to predict drug response, adverse drug responses (ADRs), dose requirements, disease susceptibility and stage. It is applicable to activities such as clinical practice, drug development and drug discovery.

DNA polymorphisms have been gaining key importance in explaining the outcome of drug treatment, as they help explain the differences in the pharmacodynamics and pharmacokinetics of drugs in different patients.

The development of new anti-hypertensive drugs and the survey of the existing ones should take into account these polymorphisms in order to adapt the drug and the drug dose to each individual and to avoid drug side effects.

In 2006, in Portugal, the estimated hypertensive population was of about 2 million people. Of these, only half knew they were hypertensive, a quarter was under pharmacotherapy and only 16% of this latter population was normotensive [(6)]. These numbers clearly show the magnitude of the problem, and because there is a considerable percentage of patients that are resistant, even to multi-drug therapy, it is believed to be important to study the contribution of individual polymorphisms to the failure of response to drug treatment.

Nevertheless, pharmacogenomics is not only useful in understanding drug response after pharmacotherapy has been initiated, it has become growingly important in drug development, enabling Stratified Medicine (pro-actively testing and selecting subsets of population for treatment based on a likely positive or negative therapeutic response). Stratification is driving a trend away from the development of "blockbuster" drugs to that of "nichebusters", which can alter the nature of competition in the pharmaceutical industry.

This can ultimately lead to Individualised Drug Therapy (IDT), also regarded as Personalized Medicine (PM), in which, genotype-based, individually targeted prescribing ought to be more effective at improving response rates and decreasing the burdens of adverse drug responses (ADRs) [(7)].

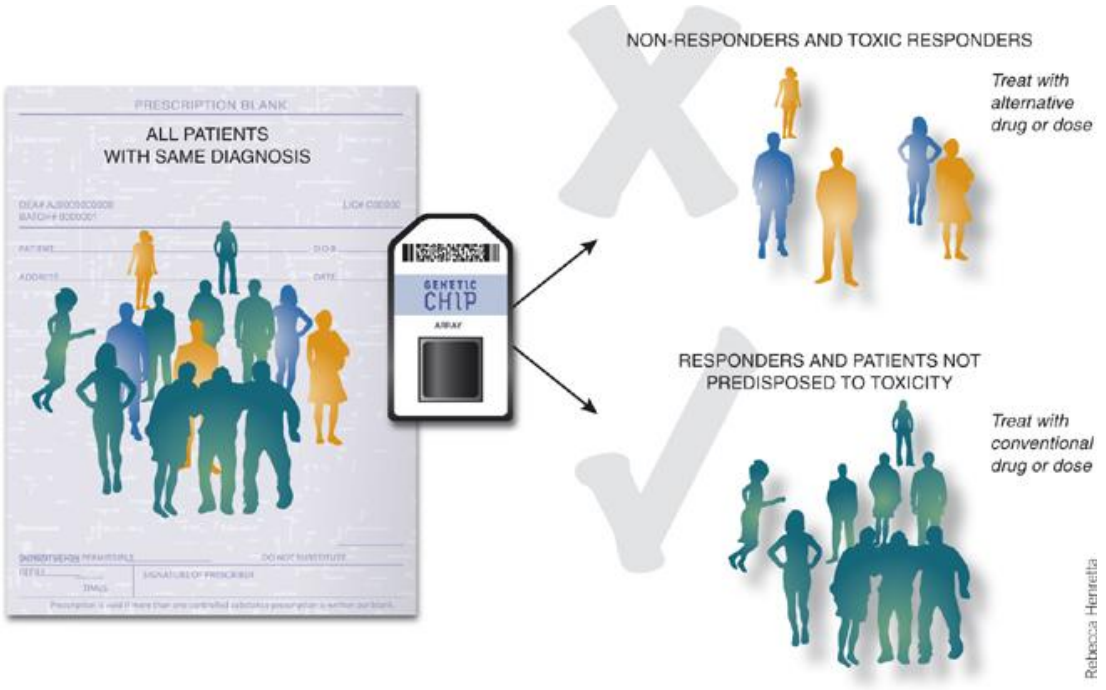

Figure 3 - Pharmacogenomic approach to Individualised Drug Therapy. Drug therapy is chosen for each patient based on their particular genetic profile. (Adapted from *The Art and Science of Personalized Medicine)*

In the case of anti-hypertensive drugs, the different classes of drugs act in different pathways or in different steps of a physio-pathologic pathway, depending on which receptor they bind to, the enzyme they inhibit, or which metabolizing step they affect. The fact that different drugs are being dealt with, amplifies the variability in response.

The guidelines proposed by the European Society of Hypertension (ESH), in its 2011 reappraisal, and the United States Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (2003) do not recommend the use of one drug over another, unless other diseases are present, such as diabetes or renal disease, once it has been shown that they do not differ significantly in their overall ability to reduce blood pressure in hypertension.

Also, because the percentage of patients responsive to any drug class is limited and patients responsive to one drug are often not those responsive to another drug, keeping the drug options large increases the chance of blood pressure control in a larger fraction of hypertensive patients. This is crucial because cardiovascular protection by anti-hypertensive treatment substantially depends on lowering blood pressure *per se*, regardless of how it is obtained [(4)].

However, not being able to control blood pressure with monotherapy leads to lower patient compliance and raises the likelihood of developing an ADR. Among other reasons, this could be avoided, as well as the side effects that lead to changing or dropping medication, if there were accurate and affordable genetic tests to overcome this problem. Thus, finding the right drug(s) to a specific individual would not be based on "trial-and-error" but on clear guidelines and algorithms of clinical care.

The available data from pharmacogenomic studies suggests that there are genetic polymorphisms that can to be taken into account when prescribing anti-hypertensive drugs. An example is the cytochrome P450 enzyme family (CYP), which has a marked influence in the pharmacokinetic (PK) phase of drug metabolism, being the genetic polymorphisms of CYP2D6 and CYP3A that play an important role in drug response for anti-hypertensive drugs. As for the pharmacodynamic (PD) phase of the cardiovascular drugs, polymorphisms in the genes encoding for α-Adducin, Angiotensin-Converting-Enzyme (ACE), Angiotensinogen and Bradykinin receptor B2 are the most important[(7)].

Pharmacogenomics is redefining how diseases are being diagnosed, classified and treated. Pharmacogenomics represent an innovative area for product differentiation, competitive advantage and research productivity enhancement. Genetic markers play a major role, in spite of the challenges of identifying valid associations and their biological complexity.

However, there are many barriers yet to overcome, such as scientific, economic, educational, legal and commercial barriers, before full potential of pharmacogenomics is achieved. Nevertheless, the scientific and technological advances in genetics and molecular science are escalating, and so does enthusiasm for the potential use of these innovations, which can significantly improve medical care delivery and health outcomes. These developments, combined with a rich array of new data and analytic tools that are easier, faster and cheaper to use, together with improved communication vehicles for health engagement, can be used to design targeted care for each individual.

Pharmacists, in all fields of action, have now another challenge to their careers: incorporating the pharmacogenomics principles into their daily practice and making use of its tools to better deliver pharmaceutical care, to develop drugs more efficiently or to discover drugs with increased safety and efficiency.

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2. Hypertension

2.1 Definition and Classification of Hypertension

Hypertension, or high arterial blood pressure, is a progressive cardiovascular disease, characterized by values of systolic blood pressure over 140mmHg and/or diastolic blood pressure over 90mmHg [1]. It is the most prevalent cardiovascular disorder in developed countries and affects *circa* 30% of the adult population [2].

Progression of the disease is strongly associated with functional and structural cardiac and vascular abnormalities that damage the heart, kidneys, brain, vasculature, and other organs, which in turn leads to premature morbidity and death.

Hypertension is classified into different categories according to the blood pressure levels. In the 2007 "*Guidelines for the Management of Arterial Hypertension*" set by the European Society of Hypertension and the European Society of Cardiology (ESH/ESC), hypertension is classified as follows:

Table 1 - Hypertension Classification

(acording to the ESH/ESC the classification is based on systolic and diastolic blood pressure)

Category	**Systolic blood pressure** (mmHg)		**Diastolic blood pressure**(mmHg)
Optimal	< 120	and	<80
Normal	120-129	and/or	80-84
High Normal	130-139	and/or	85-90
Grade 1 Hypertension	140-159	and/or	90-99
Grade 2 Hypertension	160-179	and/or	100-109
Grade 3 Hypertension	≥ 180	and/or	≥110
Isolated Systolic Hypertension	≥ 140	and	<90

The ESH/ESC guidelines also state that:

a) when a patient's systolic and diastolic blood pressure fall into different categories, the higher category should apply for the quantification of total cardiovascular risk, decision about drug treatment and estimation of treatment efficacy;

b) isolated systolic hypertension should be graded (grades 1, 2 and 3) according to the same systolic blood pressure values indicated for systolic-diastolic hypertension. However, the association with a low diastolic blood pressure should be regarded as an additional risk;

c) the threshold for hypertension (and the need for drug treatment) should be considered as flexible based on the level and profile of total cardiovascular risk. [3]

2.2 Cardiovascular Risk Assessment

Cardiovascular risk factors include hypertension and high total cholesterol, high levels of triglycerides, high levels of low-density lipoprotein or low levels of high-density lipoprotein (HDL) cholesterol.

Smoking also increases risks of cardiovascular disease. The risk is especially high for early smokers, for those who smoke heavily or for women. Stopping tobacco use can reduce the risk of cardiovascular disease significantly, despite how long a person has been smoking.

Physical inactivity increases the risk of heart disease and stroke by 50%. Obesity is a major risk for cardiovascular disease and predisposes to diabetes. Diabetes is a risk factor for cardiovascular disease.

Type 2 diabetes is a major risk factor for coronary heart disease and stroke. Having diabetes rises twofold the likelihood to develop cardiovascular disease.

Left ventricular hypertrophy is a risk factor for cardiovascular mortality.

Risk of stroke doubles every decade after age 55. If a first-degree blood relative has had coronary heart disease or stroke before the age of 55 years, for a male relative or 65 years, for a female relative, the risk increases.

The gender is significant: a male is at greater risk of heart disease than a pre-menopausal woman. But once past the menopause, a woman's risk is similar to a man's. Risk of stroke is similar for men and women.

The ethnic origin also plays a role. People with African or Asian ancestry are at higher risks of developing cardiovascular disease than other racial group.

Cardiovascular risk can, then, be stratified as follows (Table 2):

Table 2 - Cardiovascular risk stratification chart. Low, moderate, high and very high risk refers to the 10-year risk **of** a CV fatal or non-fatal event. The term 'added' indicates in all categories that risk is greater than average. The risk factors referred to in the left column are: age, smoking, dyslipidaemia, elevated fasting plasma glucose, abnormal glucose tolerance test, abdominal obesity, a family history of premature CVD and 'high pulse pressure in the elderly'. (Adapted from the 2007 Guidelines for Management of Arterial Hypertension (ESH/ESC)).

Risk factors	**Blood Pressure (Category)**				
	Normal	***High Normal***	***Grade 1 HT***	***Grade 2 HT***	***Grade 3 HT***
No other risk factor	*Average risk*	*Average risk*	*Low added risk*	*Moderate added risk*	*High added risk*
1-2 risk factors	*Low added risk*	*Low added risk*	*Moderate added risk*	*Moderate added risk*	*Very high added risk*
3 or more risk factors	*Moderate added risk*	*High added risk*	*High added risk*	*High added risk*	*Very high added risk*
Established CVD or renal disease	*Very high added risk*	*Very high added risk*	*Very high added risk*	*Very high added risk*	*Very high added risk*

This chart, among other tools, such as the Framingham Risk Score [(4)], helps to predict the risk of an individual having a CV event in the next 10 years. It is a powerful tool, available to every healthcare professional, but it does not convey the genetic information for individual DNA polymorphisms that can help decide the treatment.

2.3 Physiological Control of Blood Pressure

The human body has many mechanisms to control blood pressure: by changing the amount of blood the heart pumps, by changing the diameter of the vessels or by changing the volume of blood in the bloodstream.

To increase blood pressure the heart can pump more blood either by pumping more forcefully or more rapidly, arterioles can constrict, forcing the blood from each heartbeat through a narrower space than normal and fluid can be added to the blood stream to increase blood volume. Conversely, to decrease blood pressure, the heart can pump less forcefully or rapidly, arterioles and veins can dilate and fluid can be removed from the bloodstream.

These mechanisms are controlled by the sympathetic division of the autonomic nervous system (involuntary) and by the kidneys.

The sympathetic nervous system stimulates the adrenal gland to release the hormones epinephrine (adrenaline) and norepinephrine (noradrenaline). Both hormones raise blood pressure by causing peripheral vasoconstriction and increase cardiac output [5].

The kidneys also respond directly to changes in blood pressure. If blood pressure increases, the kidneys increase their excretion of salt and water, so the blood volume decreases and blood pressure returns to normal. On the other hand, if blood pressure falls, the kidneys decrease their excretion of salt and water so blood volume increases and blood pressure returns to normal.

Also, kidneys can raise the blood pressure by secreting an enzyme, renin, which triggers the **Renin-Angiotensin-Aldosterone System** (RAAS). In Figure 4 a schematic drawing of the RAAS is shown, where inhibitory and stimulatory pathways are depicted.

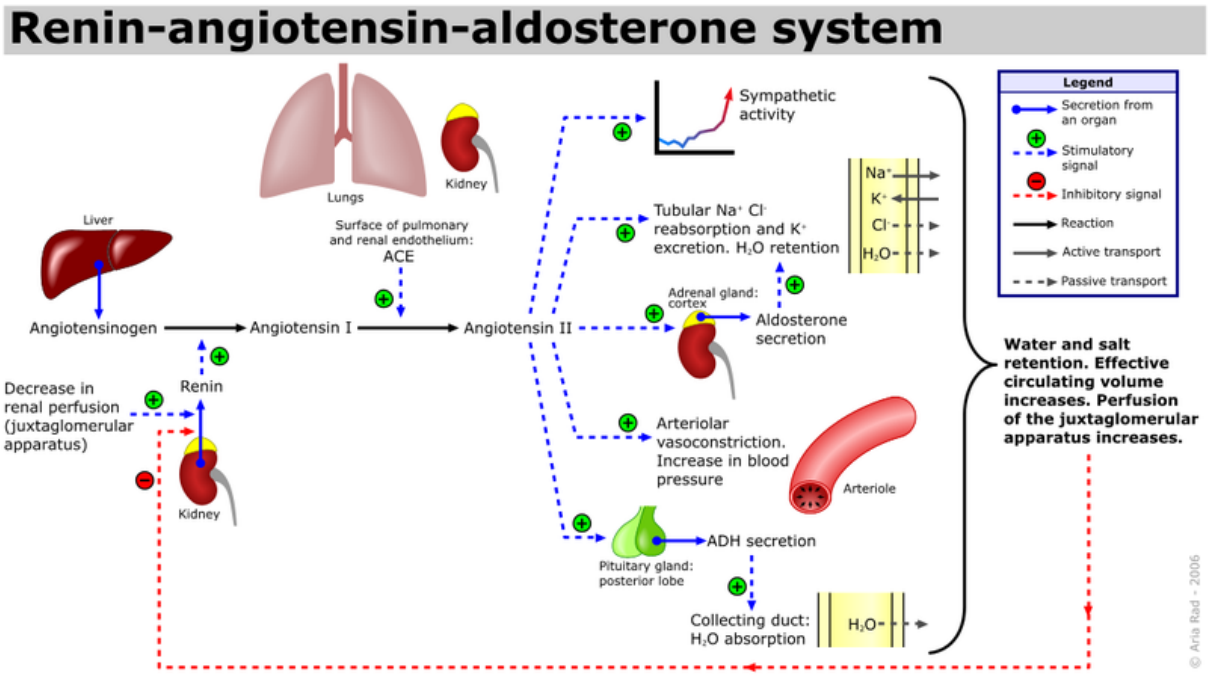

Figure 4 - Renin-Angiotensin-Aldosterone System. Blue and red dashed arrows indicate stimulatory or inhibitory signals, which is also indicated by the +/-. In the tubule and collecting duct graphics, the grey dashed arrows indicate passive transport processes, contrary to the active transport processes which are indicated by the solid grey arrows. The other solid arrows either indicate a secretion from an organ (blue, with a starting Spot) or a reaction (black). These two processes can be stimulated or inhibited by other factors. (Adapted from Wikipedia).

The RAAS plays an essential role in the homeostatic control of the arterial blood pressure, tissue perfusion and extracellular volume.

The pathway is initiated by the secretion of renin, the rate-limiting processing enzyme, from the kidney by an exocytic process involving stimulus-secretion coupling into the renal and then the systemic circulation. Renin secretion is stimulated by a fall in perfusion pressure or in NaCl delivery and by an increase in sympathetic activity.

Control of renin secretion is a key determinant of the activity of RAAS. Renin regulates the initial, rate-limiting step of the RAAS by cleaving angiotensinogen to form the biologically inert angiotensin I. The primary source of systemic circulating angiotensinogen is the liver. It's secreted constitutively by the liver, so plasma levels are generally stable. The inactive angiotensin I is hydrolysed by Angiotensin-Converting-Enzyme (ACE), to form a biological active and potent vasoconstrictor molecule: angiotensin II. ACE is a membrane-bound exopeptidase and is located on the plasma membranes of various cell types and though it also exists in a soluble form in plasma, it is the membrane-bound ACE that is thought to be physiologically important. ACE metabolizes a number of other peptides, including vasodilator peptides bradykinin and kallidin, to inactive metabolites. Thus, functionally, the enzymatic actions of ACE potentially result in increased vasoconstriction and decreased vasodilation. Angiotensin II is the primary effector of a variety of RAAS-induced physiological and pathophysiological actions. At least four angiotensin (AT) receptors have been described:

- AT1 mediates most of the established physiological and pathophysiological effects of angiotensin II. These include actions on the cardiovascular system (vasoconstriction, increased blood pressure, increased cardiac contractility, vascular and cardiac hypertrophy), kidney (renal tubular sodium reabsorption, inhibition of renin release), sympathetic nervous system and adrenal cortex (stimulation of aldosterone synthesis). It also mediates effects of angiotensin II on cell growth and proliferation, inflammatory responses and oxidative stress;
- AT2 receptor levels decrease markedly in the postnatal period but there is evidence that it might mediate vasodilatation and antiproliferative and apoptotic effects in vascular smooth muscle and inhibit growth and remodelling in heart;
- AT3 receptor's function is unknown;
- AT4 receptors are thought to modulate the endothelial function.

As stated before, angiotensin II, via AT1 receptor also stimulates the production of aldosterone. Aldosterone is a major regulator of sodium and potassium balance and thus plays a major role in regulating extracellular volume. It enhances the reabsorption of sodium and water in the kidney's distal tubules and collecting ducts and thereby promotes potassium and ion hydrogen excretion.

Deregulation of the RAAS is involved in the pathogenesis of several hypertensive disorders. In addition to the RAAS involvement in secondary forms of hypertension, there is evidence that perturbations of the RAAS are involved in essential hypertension [6].

2.4 Causes for Hypertension

Hypertension is divided into two types: primary or essential hypertension and secondary hypertension.

Primary hypertension has no identifiable cause, it is the type of hypertension with more predominance in adults and tends to develop gradually over the years.

On the other hand, some people have high blood pressure caused by an underlying condition. This type of high blood pressure, called **secondary hypertension**, tends to appear suddenly and causes higher blood pressure than does primary hypertension. Various conditions and medications can lead to secondary hypertension, including: kidney problems, adrenal gland tumours, congenital diseases, certain drugs, such as birth control pills, cold remedies, decongestants, over-the-counter pain relievers and some prescription drugs.

2.5 Pharmacological Treatment of Hypertension

Because renin is the initial and rate-limiting step in RAAS cascade, it has long been considered the logical therapeutic target for blocking the system. However, pharmacological activity of the early renin inhibitors could only be achieved with intravenous infusion, and the development of an orally active direct renin inhibitor was fraught with numerous difficulties arising from issues of potency, low bioavailability, duration of action and costs of synthesis. Since then, these obstacles have been overpassed and the renin inhibitor aliskiren is the newest anti-hypertensive molecule in the market.

Anti-hypertensive drugs are classified in several classes:

a) Beta-blockers (β-blockers);

b) Angiotensin-Converting Enzyme Inhibitors (ACEI);

c) Angiotensin II Receptor Blockers (ARB);

d) Direct Renin Inhibitors;

(all of the above act in the RAAS system.)

e) Diuretics;

f) Calcium Channel Blockers.

In the following *Table 3*, are depicted some examples of drugs that belong to the different classes presented before:

Table 3 - Examples of antihypertensive drugs, their mechanism of action and diseases in which they are used.

	Antihypertensive drugs					
	Acting in RAAS				**Diuretics**	**Calcium Channel Blockers**
	B-blockers	**ACEI**	**ARB**	**Direct Renin Inhibitors**		
Drugs (e.g.)	Atenolol Propranolol Bisoprolol	Captopril Enalapril Lisinipril Ramipril	Candesartan Irbesartan Losartan Valsartan	Aliskiren	Hydrochlotiazide Indapamide Furosemide Amiloride	Diltiazem Verapamil Amlodipine Nifedipine
Mechanism of action	*Reduces plasma renin levels by blocking sympatheticall y β-1 mediated renin release by the kidney*	*ACEIs competitively block the action of ACE and thus the conversion of Ang I to Ang II, thereby reducing circulating and local levels of Ang II*	*Specific antagonism of Ang II action at the AT1 receptor*	*Inhibits the catalytic activity of renin at the point of activation of the RAAS, blocking the synthesis of all angiotensin peptides and prevents the compensatory increase in renin activity*	*Increases excretion of water*	*Reduce muscle contractility*
	Previous stroke (any blood pressure lowering agent)					
Diseases treated	Heart failure Angina pectoris	LVH Renal dysfunction Diabetes Heart failure	Diabetes Heart failure	Primary hypertension	Heart failure	LVH Renal dysfunction Angina pectoris

It is stated, in the 2007 *Guidelines for the Management of Arterial Hypertension of the Task Force for the Management of Arterial Hypertension* [6], edited by the ESH, that "for treatment initiation, it is recommended that for Grade 1 hypertensive patients (Systolic Blood Pressure (SBP) ≥ 140mmHg or Diastolic Blood Pressure (DBP)≥ 90 mmHg), at low and moderate

risk, drug therapy should be started after a suitable time period with life changes. Prompter initiation of treatment is advisable if Grade 1 hypertension is associated with high level of risk, or if hypertension is Grade 2 or 3. In general, early blood pressure lowering treatment, before organ damage develops or becomes irreversible or cardiovascular events occur, appears a prudent recommendation, because in high risk hypertensive patients, even intense cardiovascular drug therapy, though beneficial, is nonetheless unable to lower total CV risk below the high risk threshold. On the whole, there is sufficient evidence that SBP be lowered below 140mmHg (and DBP below 90mmHg) in all hypertensive patients, both those at low moderate risk and those at high risk."

As for the choice of antihypertensive drugs, "the main benefits of antihypertensive treatment are due to lowering of BP *per se*, and are largely independent of the drugs employed. Therefore, thiazide diuretics (as well as chlorthalidone and indapamide), beta-blockers, calcium antagonists, ACE inhibitors and ARB can adequately lower blood pressure and significantly and importantly reduce CV outcomes. All these drugs are suitable for the initiation and maintenance of antihypertensive treatment either as monotherapy or in some combinations with each other. Because the percentage of patients responsive to any drug class is limited and patients responsive to one drug are often those not responsive to another drug, keeping the number of drug options large increases the chance of blood pressure control in a larger fraction of anti-hypertensive patients. This is of crucial importance because cardiovascular protection by antihypertensive treatment substantially depends on blood pressure lowering per se, regardless of how it is obtained."

Nevertheless, resistant hypertension is not a phenomenon of marginal proportions, the number of patients unable to achieve blood pressure control despite multiple drug treatment being around 15-20% [(7)]. No matter which drug is employed, monotherapy can effectively reduce blood pressure in only a limited number of hypertensive patients, most of whom require the combination of at least two drugs to achieve blood pressure control. Combining two agents from any two classes of antihypertensive drugs increases the blood pressure reduction much more than doubling the dose of one agent.

Although it is possible that the use of two drugs together implies the administration of a futile one, searching for the most effective monotherapy in every given patient is painstaking and may discourage compliance.

The avoidance of ADRs is also a matter of interest for pharmacogenomics. About 35% of hypertensive patients discontinue their medication within six months, 50% of which due to adverse effects and patient dissatisfaction [8]. One of the most common ADR is hypotension. This may be caused by overdose or overtreatment, but genomics can also be the answer if onset of action is too quick or metabolism is more extent. Hypotension is felt through symptoms such as dizziness, fatigue and anxiety. These are reasons enough to quit treatment, especially if these symptoms interfere with normal daily activities. Angiotensin-Converting-Enzyme Inhibitors can cause dry cough, which is difficult to overcome, even with drug treatment, and can disturb sleep or speech. Headaches, palpitation, flushing, alopecia and constipation are some adverse effects felt with treatment with calcium channel blockers. Beta-blockers may cause sleep disturbances, erectile dysfunction and glucose intolerance (important in diabetic patients).

These are the most perceived ADRs, others such as angioedema, renal dysfunction, hypokalaemia, hyponatremia or gout may only be diagnosed in advanced stages and, in some cases, be life-threatening.

For antihypertensive treatment, not only the costs of therapeutics are at stake, but also the costs of non-compliance and of ADRs.

In 2010, high blood pressure was projected to cost the United States $93.5 billion in health care services, medications, and missed days of work. These costs are projected to increase in the following twenty years, as can be seen in Figure 5 [9].

Whether it is by being able to choose the best drug treatment or in predicting which adverse effects will be present in a particular individual, pharmacogenomics can play a major role in defining therapeutic strategies for hypertension, cutting down health expense, improving adherence and optimizing drug therapy.

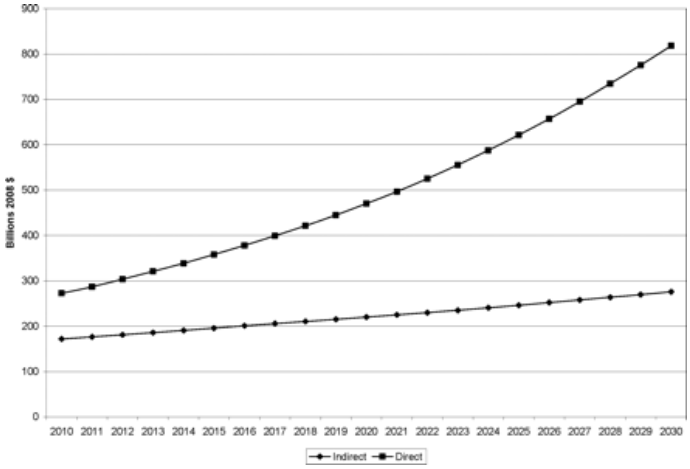

Figure 5- Projected direct and indirect costs of all CVD, 2010 to 2030 (in billions 2008$). (Adapted from *Forecasting the Future of Cardiovascular Disease in the United States*)[9].

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3. Pharmacogenomics

In April 2003 the complete sequencing of the human genome was released by the Human Genome Project. It coincided with the 50th anniversary of the research publication announcing the discovery of the DNA's double helix.

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MOLECULAR STRUCTURE OF NUCLEIC ACIDS
A Structure for Deoxyribose Nucleic Acid

WE wish to suggest a structure for the salt of deoxyribose nucleic acid (D.N.A.). This structure has novel features which are of considerable biological interest.

A structure for nucleic acid has already been proposed by Pauling and Corey[1]. They kindly made their manuscript available to us in advance of publication. Their model consists of three intertwined chains, with the phosphates near the fibre axis, and the bases on the outside. In our opinion, this structure is unsatisfactory for two reasons:

(1) We believe that the material which gives the X-ray diagrams is the salt, not the free acid. Without the acidic hydrogen atoms it is not clear what forces would hold the structure together, especially as the negatively charged phosphates near the axis will repel each other. (2) Some of the van der Waals distances appear to be too small.

Another three-chain structure has also been suggested by Fraser (in the press). In his model the phosphates are on the outside and the bases on the inside, linked together by hydrogen bonds. This structure as described is rather ill-defined, and for this reason we shall not comment on it.

We wish to put forward a radically different structure for the salt of deoxyribose nucleic acid. This structure has two helical chains each coiled round the same axis (see diagram). We have made the usual chemical assumptions, namely, that each chain consists of phosphate diester groups joining β-D-deoxyribofuranose residues with 3',5' linkages. The two chains (but not their bases) are related by a dyad perpendicular to the fibre axis. Both chains follow righthanded helices, but owing to the dyad the sequences of the atoms in the two chains run in opposite directions.

This figure is purely diagrammatic. The two ribbons symbolize the two phosphate–sugar chains, and the horizontal rods the pairs of bases holding the chains together. The vertical line marks the fibre axis

Each chain loosely resembles Furberg's[2] model No. 1; that is, the bases are on the inside of the helix and the phosphates on the outside. The configuration of the sugar and the atoms near it is close to Furberg's 'standard configuration', the sugar being roughly perpendicular to the attached base. There is a residue on each chain every 3.4 A. in the z-direction. We have assumed an angle of 36° between adjacent residues in the same chain, so that the structure repeats after 10 residues on each chain, that is, after 34 A. The distance of a phosphorus atom from the fibre axis is 10 A. As the phosphates are on the outside, cations have easy access to them.

The structure is an open one, and its water content is rather high. At lower water contents we would expect the bases to tilt so that the structure could become more compact.

The novel feature of the structure is the manner in which the two chains are held together by the purine and pyrimidine bases. The planes of the bases are perpendicular to the fibre axis. They are joined together in pairs, a single base from one chain being hydrogen-bonded to a single base from the other chain, so that the two lie side by side with identical z-co-ordinates. One of the pair must be a purine and the other a pyrimidine for bonding to occur. The hydrogen bonds are made as follows: purine position 1 to pyrimidine position 1; purine position 6 to pyrimidine position 6.

If it is assumed that the bases only occur in the structure in the most plausible tautomeric forms (that is, with the keto rather than the enol configurations) it is found that only specific pairs of bases can bond together. These pairs are: adenine (purine) with thymine (pyrimidine), and guanine (purine) with cytosine (pyrimidine).

In other words, if an adenine forms one member of a pair, on either chain, then on these assumptions the other member must be thymine; similarly for guanine and cytosine. 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 given, then the sequence on the other chain is automatically determined.

It has been found experimentally[3,4] that the ratio of the amounts of adenine to thymine, and the ratio of guanine to cytosine, are always very close to unity for deoxyribose nucleic acid.

It is probably impossible to build this structure with a ribose sugar in place of the deoxyribose, as the extra oxygen atom would make too close a van der Waals contact.

The previously published X-ray data[5,6] on deoxyribose nucleic acid are insufficient for a rigorous test of our structure. So far as we can tell, it is roughly compatible with the experimental data, but it must be regarded as unproved until it has been checked against more exact results. Some of these are given in time following, communications. We were not aware of the details of the results presented there when we devised our structure, which rests mainly though not entirely on published experimental data and stereo-chemical arguments.

It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.

Full details of the structure, including the conditions assumed in building it, together with a set of co-ordinates for the atoms, will be published elsewhere.

We are much indebted to Dr. Jerry Donohue for constant advice and criticism, especially on interatomic distances. We have also been stimulated by a knowledge of the general nature of the unpublished experimental results and ideas of Dr. *M.* H. F. Wilkins, Dr. R. E. Franklin and their co-workers at King's College, London. One of us (J.D.W.) has been aided by a fellowship from the National Foundation for Infantile Paralysis.

J.D. WATSON
F.H. C. CRICK

Medical Research Council Unit for the Study of the Molecular Structure of Biological Systems, Cavendish Laboratory, Cambridge. April 2.

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Figure 6 - James Watson (1928-) and Francis Crick (1916-2004) original paper (left). Both scientists showing a 3D mode of their proposed DNA structure (right).

Although both achievements have been hallmarks of science, in the last decade genetic research has advanced from the human genome sequencing to the mapping of genetic variations among individuals. Recently, these individual genetic variations have been associated with and used to identify diseases and predict drug response and adverse reaction.

Each individual has a particular drug response, ranging from high efficacy and no toxicity to no efficacy and high toxicity. This drug response depends on several factors, such as: gender, lifestyle, age, race, disease severity, disease progression, underlying illnesses, concomitant drug therapy and the genetic makeup.

For the last century there has been increasing evidence that how individuals respond to drugs varies, to some extent, according to inherited characteristics and are therefore genetic bound. These inter-individual genetic variations that result in different drug responses are studied in a fairly new and fast growing field of science: **pharmacogenomics**.

3.1 History of Pharmacogenomics

It has been almost 150 years since the publication of Gregor Mendel's "Versuche über Planzenhybriden" (Experiments in plant hybridization, 1865), the hallmark of genetics. In this brilliant paper, Mendel brought forward the principles of heredity, introduced us to the meaning of dominant and recessive characteristics and was the first to apply mathematics and statistics to a biological problem [(1)].

Six years before Charles Darwin had published "On the Origins of Species" (1859) and four years later DNA was isolated (Miesher, 1869).

In 1902, Archibal E. Garrod published in "The Lancet" a paper where he found that "there are good reasons for thinking that alkaptonuria is not the manifestation of a disease, is rather of the nature of an alternative course of metabolism, harmless and usually congenital and lifelong." [(2)]. This is considered to be the first pharmacogenetics paper ever written, though the term "pharmacogenetics" would only make its appearance in 1959, through Vogel's work ("Modern Problem der Humangenetic") in which he described this new scientific discipline that dealt with inherited differences in response to drugs.

In 1962, Kalow W. published the first book in the field: "Pharmacogenetics: Heredity and the response to drugs".

Through the years, along with the unveiling of the complete human genome sequence, genetic testing has evolved from the study of rare monogenic diseases to more common and genetically complex diseases such as cancer or cardiovascular disorders. Nowadays

pharmacogenomic testing has been the focus of many scientist's work, who try to find the answer to how DNA polymorphisms can be related to drug response.

3.2 Definitions

The **genome** of an organism comprises the entire DNA that can be found in a cell (nuclear and mitochondrial). Within this DNA are genes that function to build and maintain the organism, and *circa* 95% of the genes are transcribed into messenger ribonucleic acid (mRNA) which ultimately are translated into proteins.

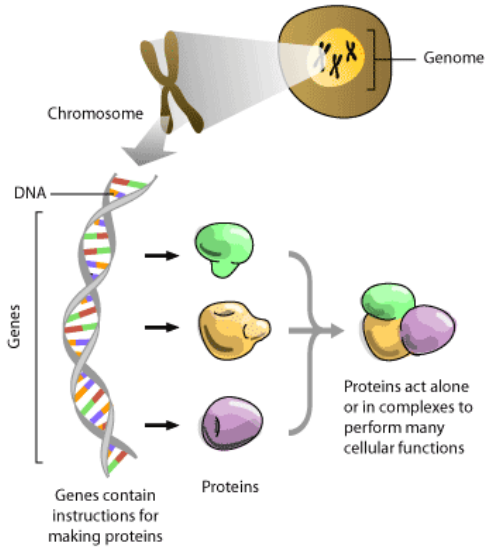

Figure 7- From genes to proteins (Adapted from http://www.scq.ubc.ca/the-human-genome-project-the-impact-of-genome-sequencing-technology-on-human-health/).

Genomics study the structure of genes (how they are organized and how that organization is relevant to gene regulation and function), the content of the genome and the evolution of genomes. The genome size and complexity vary greatly among organism, for example, the human genome is made of about 23.000 genes, with 3.000.000 of base pairs, while the rat genome has 25,557 genes, with 275.000 of base pairs.

With so many base pairs (and genes), the human genome is greatly diverse, especially if it is taken into account the copying errors that can occur during DNA replication. With each generation, the genetic material contained in the chromosomes must be copied. It is during this copying process that mutations can occur in the DNA sequence. These changes

ultimately give rise to the diversity of life…and of drug response (the object of study of PGx).

Mutations can encompass changes from a single base pair substitution (single nucleotide polymorphisms (SNPs)), to de addition or loss of DNA.

Aside from approximately 23.000 functioning genes, humans have nearly as much of non-functioning pseudogenes. Of the functioning genes, 5 to 10% are RNA genes. The remaining genes are transcribed into mRNA and translated into proteins.

Gene's nomenclature is also important to understand. Gene names are determined by the Human Genome Organization that approves an unique name and symbol for each gene, so it can be clearly perceived by all. Gene names are generally short and intended to convey some information as to their function. Members of the same gene superfamily share the same root name (e.g., cytochrome P450 superfamily gene all start with CYP), and Arabic numerals are used to distinguish individual members of the family (examples in *Table 4*). Given that in humans the rate of mutations is approximately 2,5 changes in 10^8 sites per generation, per gene, there are many variants. These variants (or alleles), are designated by the gene name followed by an asterisk and an Arabic numeral, for example: CYP3A4*2 is one allele of at least 20 known alleles in this gene [3].

Table 4 - Gene nomenclature (examples).

Approved Symbol	Approved Name	Chromosome location
CYP1A1	cytochrome P450, family 1, subfamily A, polypeptide 1	15q24.1
CYP2A6	Cytochrome P450, family 2, subfamily A, polypeptide 6	19q13.2
VKORC1	Vitamin K epoxide reductase complex, subunit 1	16p11.2
NAT6	N-acetyltransferase 6 (GCN5-related)	3p21.3
UGT1A7	UDP glucuronosyltransferase 1 family, polypeptide A7	2q37

Pharmacogenetics and pharmacogenomics are two complementary principles. As defined by the WHO: "Pharmacogenomics refers to the study of DNA sequence variation as it relates to differential drug response in individuals, i.e., the use of genomics to determine an individual's response. Pharmacogenetics refers to the use of DNA-based genotyping, in order to target pharmaceutical agents to specific patient populations in the design of drugs" (*WHO Drug Information, Vol 16, No.1, 2002*). But, both terms can be used interchangeably [3] and the term pharmacogenomics will be used hereafter.

Pharmacogenomics research encompasses the use of appropriate DNA methodologies to develop reliable biomarkers to predict drug response, ADRs, dose requirements, disease susceptibility and stage. It is applicable to activities such as clinical practice, drug development and drug discovery.

After administration, the drug response depends on its disposition (pharmacokinetics, PK) and effect (pharmacodynamics, PD).

Pharmacokinetic effects are due to inter-individual differences in absorption, distribution, metabolism, or excretion (ADME) of the drug. Inappropriate concentration of the drug, existence of inappropriate metabolites or both can result in a lack of efficacy or in toxicity. Such PK effects are linked mainly to the cytochrome P450 enzyme family, but also to membrane transporters or other metabolizing enzymes.

Variations in ADME genes can result in the absence of a protein or the production of a protein with altered or no activity (including transporters, enzymes and drug targets). There have been found many variations in genes accounting for the variation in plasma drug concentration, such as mutations in the CYP2D6. The clinical significance of these variations depends primarily on the contribution of the specific pathway to the overall metabolism of the drug, as well as the activity of its metabolites.

Pharmacodynamic effects may lead to inter-individual differences in a drug response, albeit the presence of appropriate drug concentrations at the intended site of action. DNA-based variation in the target molecule's gene or downstream mechanistic pathway genes may also explain the variability among subjects in drug response [3] (Figure 8).

Nowadays PGx is not only applied in the study of response to drugs but also as a guide to drug development, as the pharmaceutical industry searches for innovation, increased productivity, ways to better differentiate from competitors, and ways to bring better, safer, more efficient drugs to market with lower costs of development.

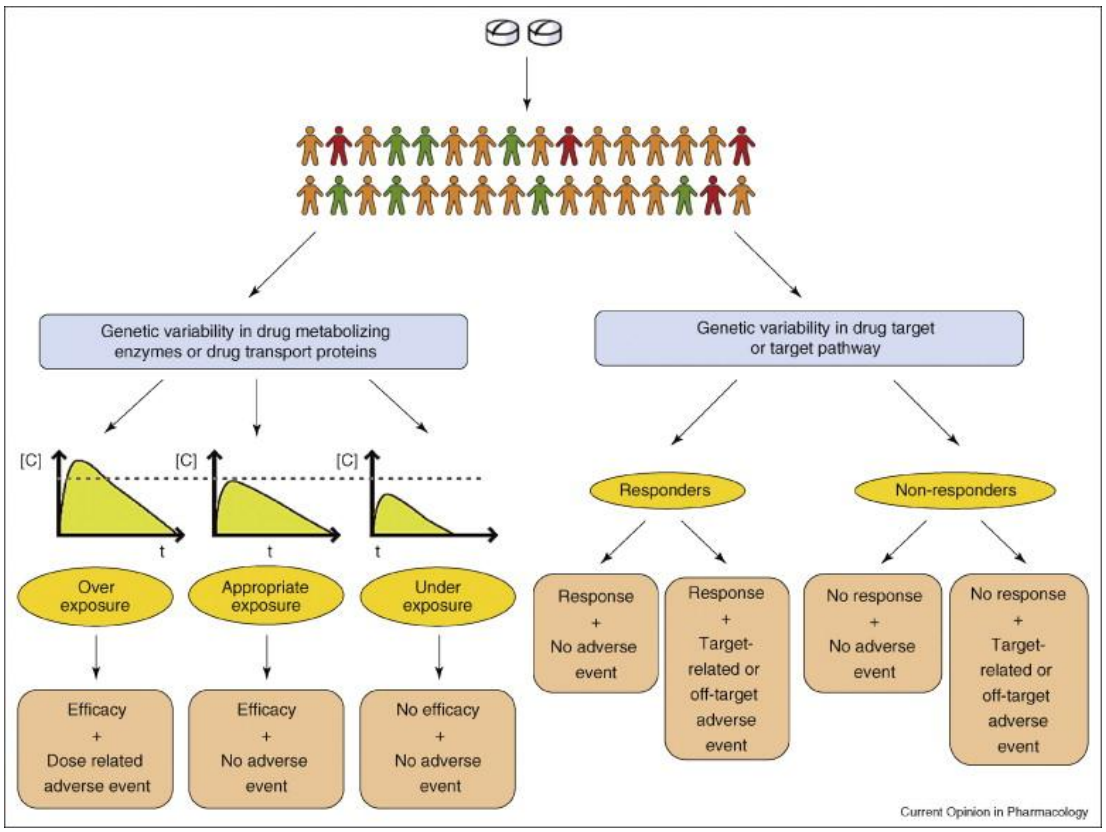

Figure 8- A schematic overview of genetic variability affecting drug response and disposition. [C] is the drug concentration and *t* is time. (Adapted from Bathenha A, Pharmacogenetics: improving drug and dose selection).

In January 2012, the Committee for Medicinal Products for Human Use (CHMP) adopted the Guideline on the use of PGx methodologies in the pharmacokinetic evaluation of medicinal products, proposed by the European Medicines Agency (EMA). The aim of this guideline is "to clarify the requirements related to the use of pharmacogenetics in the pharmacokinetic evaluation of medicinal products". It "addresses the influence of pharmacogenetics on drug pharmacokinetics, encompassing considerations and requirements for design and conduct investigations during drug development". This guideline considers of high importance the existence of pharmacogenetics/pharmacogenomics studies where they play a key role in establishing the benefit-risk of a drug in the different stages of drug development.

In a clinical setting, the application of pharmacogenomics to an individual can be referred to as Individualised Drug Therapy (IDT). This ability to offer the right treatment to the right person as needed, with pharmacogenomics-based individualized pharmacotherapy is its ultimate goal. But not only the genetic makeup must be taken into account, the clinical status and the environmental information are also of importance. And being able to translate

these findings into precise diagnostic tests and targeted therapies is what scientists, physicians and patient are longing for.

There are many advantages that IDT offers, to both doctors and patients, such as the ability to make more informed medical decisions, higher probability of desired outcomes given better targeted therapies, reduced probability of negative ADRs, focusing on prevention and prediction disease rather than reaction to it, earlier disease intervention and reduced healthcare costs [(4)].

Another term is often used – **stratified medicine** – which means pro-actively testing and selecting subsets of populations for treatment based on a likely positive or negative therapeutic response. Stratification is driving a trend away from the development of "blockbuster" drugs to that of "nichebuster", which could ultimately alter the nature of competition in the pharmaceutical industry [(3)].

Also important is the definition of **genomic biomarker**: "a measurable DNA and/or RNA characteristic that is an indicator of normal biologic processes, pathogenic processes, and/or response to therapeutic or other interventions." [(6)]. Many of the pharmacogenomics research includes the identification of these biomarkers and then translating this information into predictive tests for further use in clinical or drug development settings.

3.3 Genomic Technologies

Technological advances in the past decade, in the field of genomics, such as fast DNA sequencers or high-throughput DNA screening, have facilitated rapid biomarker discovery, the unravelling of new targets and enabled mechanistic studies that helped to understand the drug's mechanisms of action.

The discovery of the cellular tools necessary to cut and restrict DNA initiated the revolution in molecular biology. On this discovery a Nobel Prize was awarded to Werner Arber, Dan Nathans and Hamilton Smith, in 1978, for "the discovery of **restriction enzymes** (or endonucleases) and their application to problems of molecular genetics"[(6)].

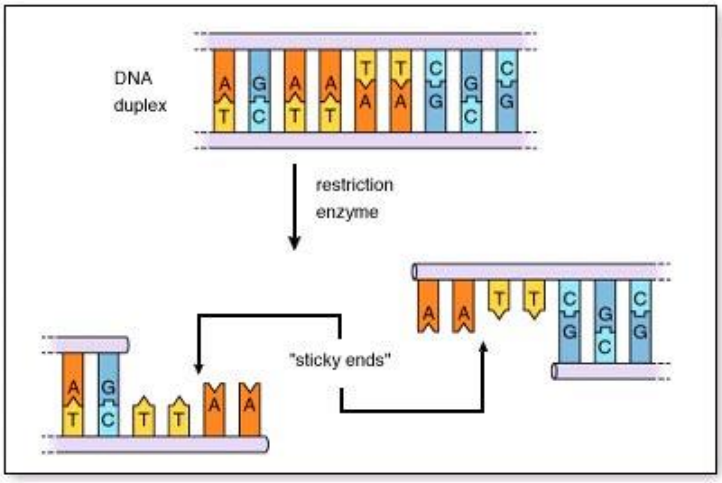

Figure 9 - **Restriction Enzymes**. Given the size of the genome, the ability to reliably and repeatedly cut DNA at specific locations and recover specific fragments with known sequences at each end, allows scientists to part the genome in sections small enough to study. The restriction enzymes are isolated from bacteria and recognize short sequences of double-strand DNA, which they cut leaving behind sticky ends (as in Figure 9) or blunt ends. With the ability to reliably produce DNA fragments with known sticky ends, it is possible to recombine fragments by using overlapping ends to facilitate the joining of the DNA fragments. Typically, DNA fragments are ligated into specialized plasmids as vectors for the DNA fragment to allow further study, replication or sequencing. (Adapted from http://www.tumblr.com/tagged/process?before=1352065342).

The next important step was to discover a method to determine the sequence of DNA.

In 1977, Frederic Sanger proposed a method to sequence the DNA – Sanger Sequencing Method – that is based on the use of dideoxynucleotides (ddNTP's) in addition to the normal nucleotides (NTP's) found in DNA. Dideoxynucleotides are essentially the same as nucleotides except they contain a hydrogen group on the 3' carbon instead of a hydroxyl group (OH). These modified nucleotides, when integrated into a sequence, prevent the addition of further nucleotides. .This occurs because a phosphodiester bond cannot form between the dideoxynucleotide and the next incoming nucleotide, and thus the DNA chain is terminated.

The Nobel Prize in Chemistry was awarded to him in 1980 in this discovery, to be added to a former one, in 1958, for his work on the structure of proteins, especially that of insulin.

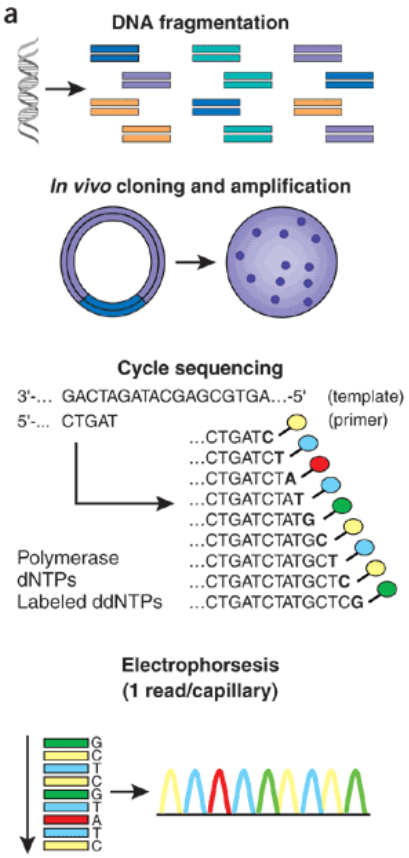

Figure 10 –Sanger Sequencing Method -The classical chain-termination method requires a single-stranded DNA template, a DNA primer, a DNA polymerase, normal deoxynucleotidetriphosphates (dNTPs), and modified nucleotides (dideoxyNTPs) that terminate DNA strand elongation. These chain-terminating nucleotides lack a 3'-OH group required for the formation of a phosphodiester bond between two nucleotides, causing DNA polymerase to cease extension of DNA when a ddNTP is incorporated. The ddNTPs may be radioactively or fluorescently labeled for detection in automated sequencing machines. The DNA sample is divided into four separate sequencing reactions, containing all four of the standard deoxynucleotides (dATP, dGTP, dCTP and dTTP) and the DNA polymerase. To each reaction is added only one of the four dideoxynucleotides (ddATP, ddGTP, ddCTP, or ddTTP). Following rounds of template DNA extension from the bound primer, the resulting DNA fragments are heat denatured and separated by size using gel electrophoresis. This is frequently performed using a denaturing polyacrylamide-urea gel with each of the four reactions run in one of four individual lanes (lanes A, T, G, C). The DNA bands may then be visualized by autoradiography or UV light and the DNA sequence can be directly read off the X-ray film or gel image. Technical variations of chain-termination sequencing include tagging with nucleotides containing radioactive phosphorus for radiolabelling, or using a primer labeled at the 5' end with a fluorescent dye. (Adapted from http://www.nature.com/nbt/journal/v26/n10/fig_tab/nbt1486_F1.html).

In the 1980's another advance in molecular biology made it possible to amplify a specific DNA fragment by a billion-fold within a heterogeneous mix of DNA: **Polymerase Chain Reaction** (PCR). Again this discovery led to a Nobel Prize in Chemistry awarded to Michael Smith, in 1993.

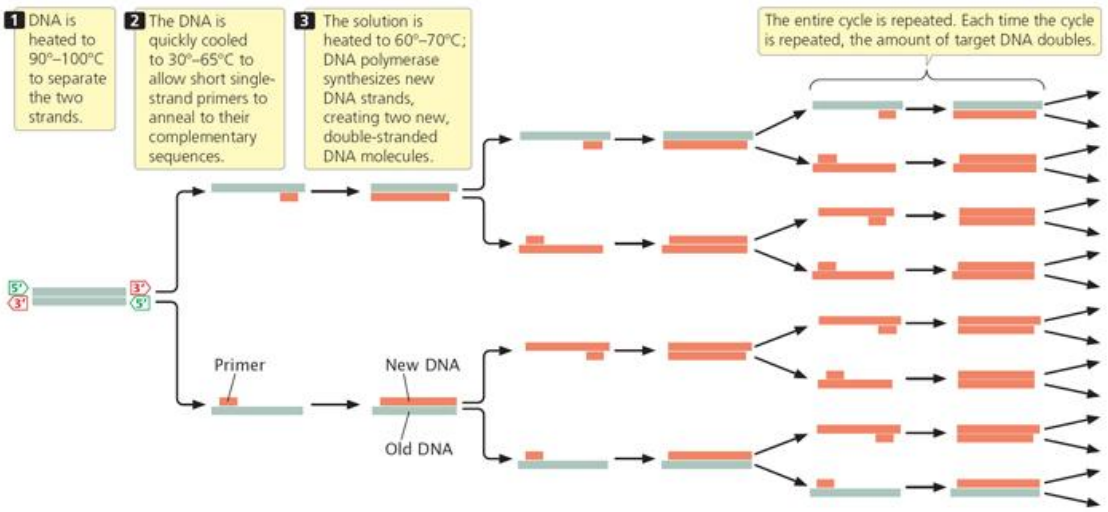

Figure 11 - Polymerase Chain Reaction: it employs short, specific primers that anneal to the opposing strands of denatured double-strand DNA and a DNA polymerase to synthesize new complementary strands. This cycle can be repeated several times. The primers are designed to be complementary and specific to the region which is to be amplified. (Adapted from http://www.nature.com/scitable/content/using-polymerase-chain-reaction-to-amplify-even-19492).

These three tools, restriction enzymes, Sanger sequencing and PCR, were the ground pillars for the development of many genomic technologies that are used nowadays: next-generation sequencing (NGS) systems, DNA microarrays and Real-Time PCR.

The **NGS** comprise the massively parallel DNA sequencing that can generate in a single experiment a great amount of sequenced DNA. These technologies use miniaturized and parallelized platforms for sequencing of 1–100 million of short reads (50-400 bases). NGS parallelization of the sequencing reactions generates hundreds of megabases to gigabases of nucleotide sequence reads in a single instrument run. This has enabled a drastic increase in available sequence data and fundamentally changed genome sequencing approaches in the biomedical sciences. Newly emerging NGS technologies and instruments have further contributed to a significant decrease in the cost of sequencing nearing the mark of $1000 per genome sequencing [8]. Examples of these NGS platforms are the Roche 454® or Illumina®.

The **DNA microarray** (Figure 12) or biochip is a collection of microscopic DNA features attached to a solid support (glass, plastic or silicon). They allow the assessment of DNA segments that are present in a given sample. In the pharmaceutical research, the microarrays are used to address three basic questions: gene expression, genetic tests and comparative genomic hybridization.

Pharmacogenomic tests can use these microarrays as gene expression tools, as they can give insight to the underlying mechanisms of drug response, and as genetic tests, as they may be composed of a battery of potential SNPs.

Array comparative genomic hybridization has, in the past few years, superseded traditional chromosome-based methods for the detection of genomic copy number variations. It permits higher resolution levels than chromosome-based methods, facilitating identification of chromosomal changes, such as microdeletions and duplications [9].

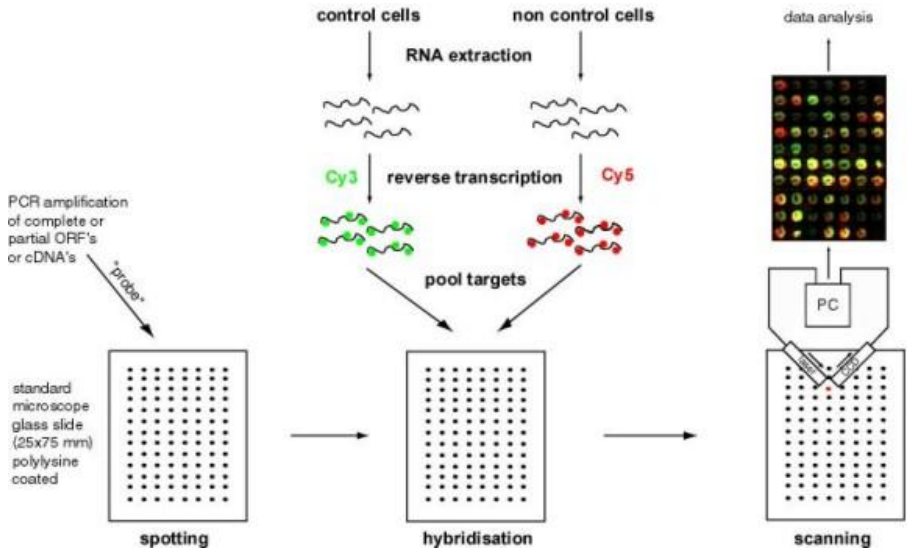

Figure 12 - Principles of DNA microarray: the two complementary sequences of DNA will bind (hybridize) with each other. Thus if one half of the DNA sequence is covalently bound to a support (array) and its complement is present in a sample to be assayed, the two will bind to one another. Typically, the sample of DNA sequences to be assayed is labelled using an isotope, fluorescent dye or chemiluminescence. These arrays yield image files containing intensity values resulting from the hybridization of the labelled DNA to the targets on the array. (Adapted from http://www.uni-koeln.de/med-fak/biochemie/transcriptomics/07_analysis.shtml).

Real-time PCR allows the assessment of specific quantities of specific targets with the ability to monitor them in real time. Through the use of DNA dyes or fluorogenic probes, the amount of DNA accumulating in each cycle of the PCR amplification process can be quantified in real time. This way researcher can estimate the initial starting concentration of a given target. It is a faster, less expensive and more quantitative process than microarrays. On the other hand, with this technic, the number of genes that can be assayed is much less than in microarrays.

3.4 Current applications

As stated before, the field of PGx includes the application of genomic technologies to identify networks of genes that affect drug efficacy and toxicity, ascertain new therapeutic drug targets and optimize current pharmacotherapeutic treatments.

This means that PGx has enormous potential in the different steps of drug development. It can [4]:

(a) Improve the discovery of targeted drugs;

(b) Improve proof of principle for efficacy trials (and salvage drugs);

(c) Identify optimal dosing;

(d) Improve drug safety and understand adverse events in the development and post-approval;

(e) Improve the identification of patients who will benefit from genetically-defined therapy, thereby avoiding futile therapeutic attempts.

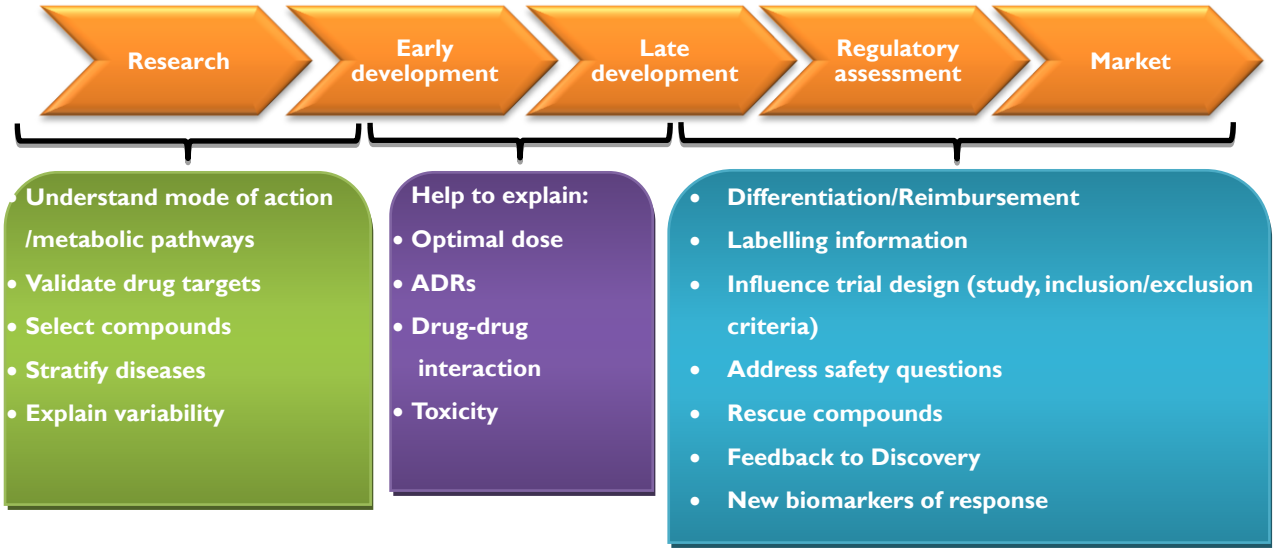

Figure 13 - The use of PGx studies in the pharmaceutical lifecycle. Throughout the life cycle of a drug PGx studies can be made in order to enhance each step of the way. In orange are the different steps in drug discovery and development and below are depicted the uses of PGx studies in each phase. **(**Adapted from Pharmacogenomics and personalized Medicine, 2008).

As seen in Figure 13, pharmacogenomic studies can be applied throughout the process of drug discovery, development and commercialization. From the research phase to the drug's commercialization, the need for PGx testing exists and is well accounted for.

Currently, there are several pharmacogenomic tests available. The United States Food and Drug Administration (FDA) provides a list of pharmacogenomic biomarkers depicted in drug labels which, if performed (some being compulsory, like for certain oncologic drugs), will allow a better drug dosage or avoid ADRs. In the list bellow (Table 5) it can be found examples of drugs with labelled PGx biomarkers, used in cardiovascular diseases:

Table 5 - Table of Pharmacogenomic Biomarkers in Drug Labels of Cardiovascular Drugs (Adapted from http://www.fda.gov/drugs/scienceresearch/researchareas/pharmacogenetics/ucm083378.htm).

Drug	Therapeutic Area	Biomarker
Carvedilol	Cardiovascular	CYP2D6
Clopidogrel	Cardiovascular	CYP2C19
Isosorbide, Hydralazine	Cardiovascular	NAT1; NAT2
Metoprolol	Cardiovascular	CYP2D6
Propafenone	Cardiovascular	CYP2D6
Propranolol	Cardiovascular	CYP2D6

Recently, the development of drugs that depend on the use of a genomic test to meet their labelled safety and efficacy claims, has become more common (in the FDA's *Table of Pharmacogenomic Biomarkers in Drug Labels* there are circa 100 drugs listed). Such tests can identify appropriate subpopulations for treatment or identify populations who should not receive a particular treatment because of an increased risk of a serious ADR.

In Portugal, the *Centro de Genética Clínica (http://www.cgcgenetics.com)* provides a cardiovascular pharmacogenomic test (among others) that allows drug dosage adjustment. Based on the test result a patient can be classified as poor metabolizer, extensive metabolizer or intermediate metabolizer and the drug dose can then be titrated or the drug can be replaced if it is found to be ineffective in a particular patient. This test requires information

about the ordering physician and the signing of an informed consent which reads: "*I wish to make the tests above indicated, and I assure that I was properly and fully informed, so I give my consent. I also authorize the report to be sent to my physician and my sample to be used for research purposes.*"

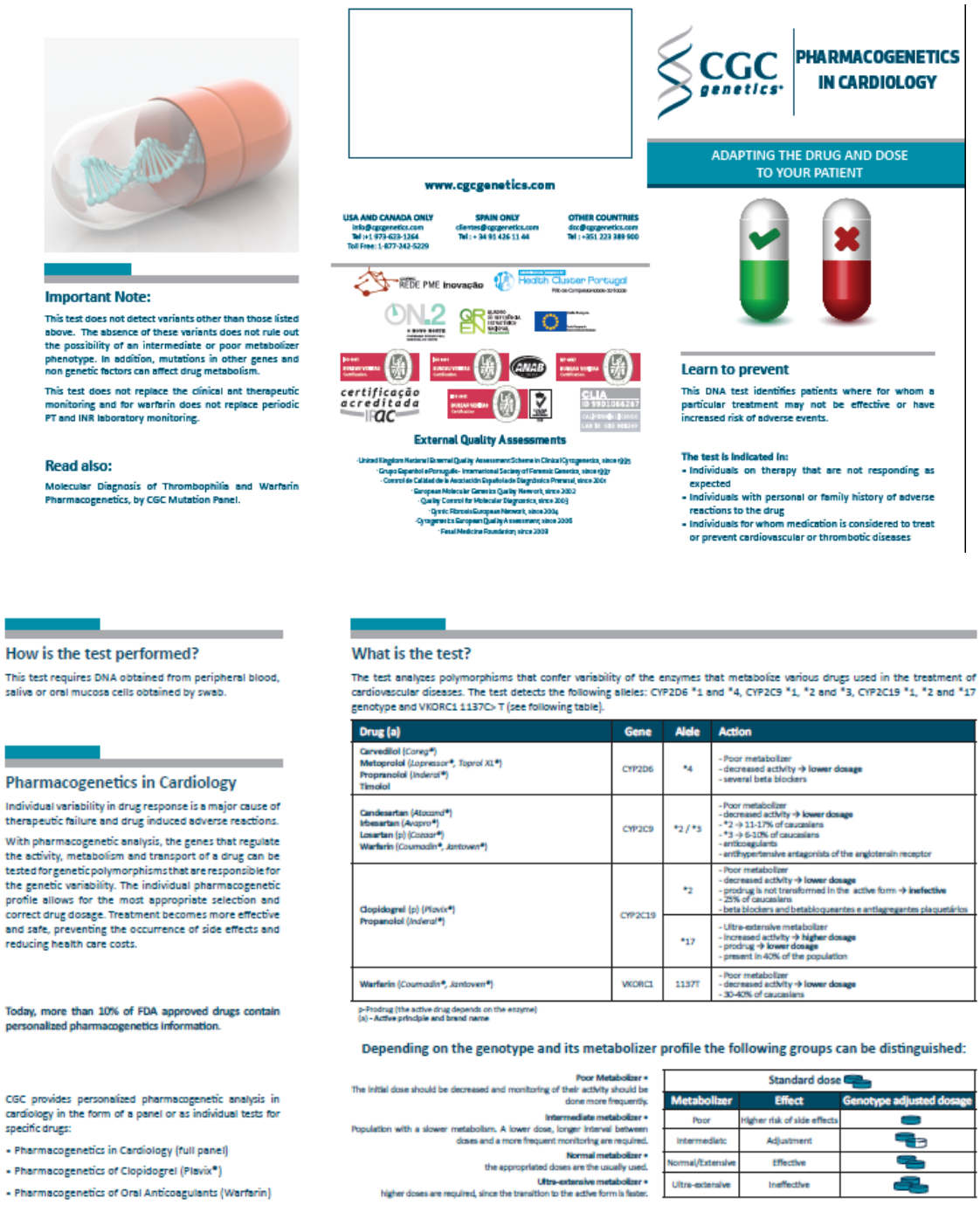

Figure 14 - Pharmacogenomic test performed by CGC (Extract from a leaflet advertising the cardiovascular pharmacogenomic tests performed by CGC. (Adapted from http://www.cgcgenetics.com/cgc/en/main-en.html).

3.5 Pharmacogenomics and the Pharmaceutical Industry

The benefit of pharmacogenomic testing in a clinical setting is clear: the right drug for a specific disease afflicting a particular individual. But how clear are the benefits for the pharmaceutical industry?

There are many questions regarding the implementation of PGx testing in the pharmaceutical industry and how it may add value to the discovery and development of drugs. The loss of the blockbuster at the hands of pharmacogenomics is a key factor, but although there are risks like the loss of patients who are at risk of ADRs and the loss of non-responders, there are also many benefits.

The pharmaceutical industry has to gain with the possibility of an earlier market introduction of a drug, with faster approvals, recruiting patients to treat new subtypes of a disease, earlier/preventive use of drugs, enhanced patient compliance, and potential higher pricing and reimbursement for best-in-class drugs.

Any concerns about the limited market resulting from drug efficacy only in patient subgroups could be assessed by exploring a broader group of patients once ideal proof-of-efficacy has been achieved.

The pharmaceutical industry operates in a highly regulated environment, inspected by health authorities such as the United States FDA and the European Medicines Agency (EMA), and it has obligations to its stakeholders, such as patients, physicians and stockholders. The introduction of PGx studies may present benefits and also risks that need to weighed.

Nevertheless, the application of PGx testing is already on the way and becoming a transversal issue in the all process of pharmaceutical life-cycle, as can be seen in Figure 15.

In drug discovery, pharmacogenomic testing can be found from the very start of the process, with the choice of the target, the choice of the chemical lead, the pre-clinical testing and clinical development, onto the identification of new compounds successfully transitioned into the clinic [(4)].

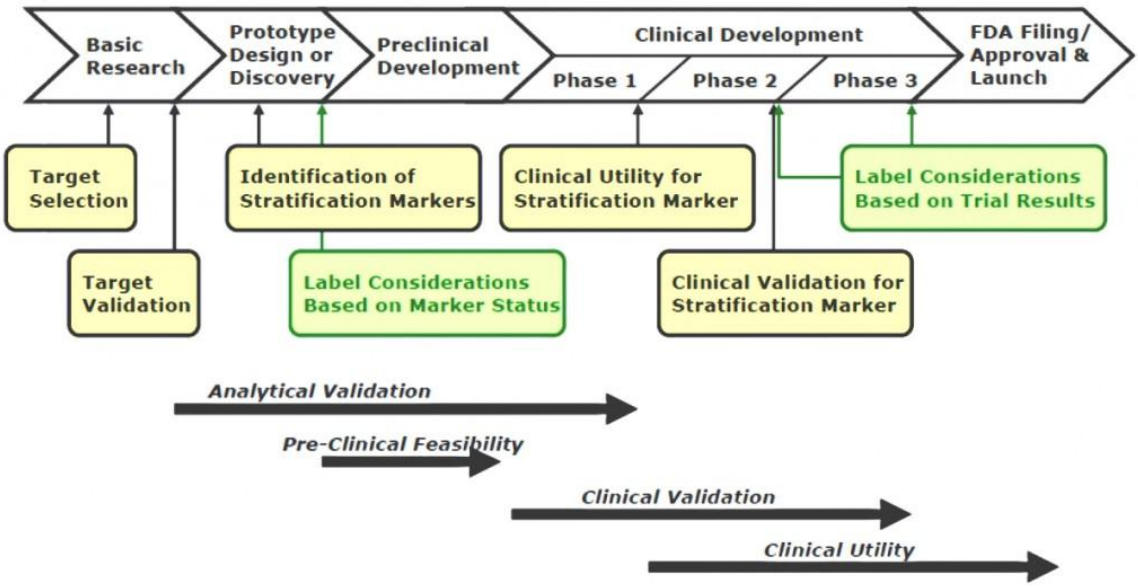

Figure 15 - Biomarkers use during drug discovery and development. The development of predictive safety and efficacy biomarkers are expected to reduce the time and cost of drug development. The use of biomarkers can further facilitate decision-making through preclinical development and into clinical trials. (Adapted from FDA Drug-diagnostic Co-development draft concept paper – 2005).

Specifically, in an exploratory study for target selection and toxicity prediction, PGx is useful for patient segmentation (drug efficacy) and can be specific for each individual (drug toxicity). However, in areas such as oncology, efficacy PGx can be used more specifically to select patients where ADRs must be avoided, in those in whom there is little chance of efficacy.

Early efficacy PGx (phase I and II) can also lay the foundations for identifying patients who require a different drug regimen. The ability to identify subgroups of patients responding in a different way to compounds during phase II development could permit the progression of multiple compounds for the same therapeutic area with patients being stratified to the compound by genotype[9].

The European regulatory health authority (EMA) has recommendations on how to implement pharmacogenomics during the different phases of clinical development, starting with the in vitro studies conducted before investigation of the medicinal product in man laid down in its "*Guideline on the use of pharmacogenomic methodologies in the pharmacokinetic evaluation of medicinal products*"(December 2011).

The pharmaceutical industry is adapting itself to this new paradigm, not only in its way of thinking the drug development process, but also in its infrastructures.

The key elements to consider when establishing a pharmacogenomics infrastructure in a pharmaceutical industry setting are the following [4]:

- Develop stringent procedures to enable routine collection of samples in clinical trials and either the de-identification or anonymization of the samples for long-term storage to ensure the privacy and confidentiality of the subjects. Develop informed consent forms and pharmacogenomics protocol templates which meet high ethical and regulatory standards;
- Develop laboratory capabilities, including a biobank, with stringent and standardized procedures and an adequate data management system to store information about DNA samples, genotyping and clinical data;
- Develop bioinformatics and biostatistics capabilities to create tool to mine and analyse the genetic data and to generate reports;
- Develop a pharmacogenomics strategy as a part of a clinical development plan involving a multifunctional team – pharmacogenomics and discovery scientists, clinical pharmacologists, physicians, statistics/bioinformatics specialists, commercial/marketing experts, and regulatory affairs and diagnostic consultants;
- Pharmacogenomics implementation:

  a. collecting samples in all trials is highly recommended;

  b. where possible, use genomics information to guide trial design;

  c. include pharmacogenomics as early as possible in the overall development program and examine the feasibility of linkage of the drug to a companion diagnostic as early as possible;

  d. where candidate gene approach is inadequate, evaluate the feasibility of a genome wide screen approach;

  e. do not limit application of pharmacogenomics to biomarker identification. Exploit pharmacogenomics to identify or support mode of action in vivo, and to optimize discovery processes and clinical trials;

  f. consider both DNA and RNA markers, as well as other types of markers (imaging, proteomics) when appropriate;

  g. pharmacogenomics in clinical trials should be a balancing act of experiments where hypothesis are being generated and tested.

The use of pharmacogenomics in drug discovery can improve the decision-making process and provide substantial cost savings by reducing timelines and promoting the judicious allocation of resources. In drug discovery, genetic variability analysis should be performed as soon a prospective target is identified. Potential benefits of early analysis include avoiding targets with unmanageable variability, and selecting the variant(s) that are most prevalent in human populations, thus improving the likelihood of success in clinical trials.

Once a compound is adequately characterized, early integration of pharmacogenomics biomarkers into development can ideally lead to faster development, approval, adoption and penetration, and will in turn lead to commercial advantages that will outweigh the disadvantages of smaller eligible patient populations. Additionally, earlier opportunity assessment can facilitate a company's potential to adequately differentiate a compound and strengthen its value proposition for pricing and reimbursement authorities [11].

Pharmacogenomics is challenging for both pharmaceutical development and commercialization. It is an innovative area of knowledge that allows product differentiation, competitive advantages and Research & Development (R&D) productivity enhancement. The regulatory agencies are up-to-date with these challenging changes and so is science. The discovery of reliable genetic markers is also highly important, in spite of the difficulty of identification of valid associations with the drug response.

The development of PGx science and technology, applicable to the individualised drug therapy is proceeding at a rapid pace, such as it not a matter of "if" it could be put to action, but rather "when" it will become part of a clinical routine, leading to a major impact in worldwide healthcare.

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4. Pharmacogenomics and Hypertension

Cardiovascular diseases are the major cause of death in the developed world. They are a large group of multifactorial pathologies in which hypertension is present in a large number of these diseases. Except for particular cases, such as existence of concomitant pathologies, hypertension can be treated with a variety of drugs, independently of which, as long as lowering blood pressure is obtained. Nevertheless, *circa* 15% of all patients do not respond to any treatment, even those that comprise the use of two or more drug combination. One of the reasons for this resistance is the genetic variability between individuals [1].

There have been identified groups of genes that are involved in these different responses [1]:

1. Genes regulating the **pharmacokinetics** phase of the drug: Cytochromes P450: CYP2D6 and CYP2C9-C19 are the most frequently involved (captopril, diltiazem or nifedipine are some examples). However, transporters like those of the ABC family also have been taken into account (atenolol or losartan);
2. Genes related to the **pharmacodynamics** phase: the enzymes inhibited by the drugs (e.g., angiotensin converting enzyme) and the receptors involved in their actions (beta-adrenergic receptors, angiotensin I and II receptors) are also important pharmacogenomic targets;
3. Gene polymorphisms from the **patients under treatment** related to the pathology introduce a third range of pharmacological variability (e.g., inflammation pathway genes);
4. Health and physiological mechanisms (e.g., age, gender, ethnicity, weight) could also modify the gene polymorphisms' effect;
5. Environmentally sensitive genes are important and interfere in the pharmacogenomic response of many drugs.

Ideally, the development of new antihypertensive drugs and the choice of the existing one for clinical use, should take into account these polymorphisms in order to adapt the drug dosage to each individual and to avoid side effects.

Drug's efficacy and toxicity can be affected by both pharmacokinetic and pharmacodynamics related polymorphic genes.

Pharmacokinetic related genes:

• a large number of cardiovascular drugs are lipophilic, which means that the polymorphism in the genes related to the absorption, distribution, metabolism and elimination have a high impact on the response to these drugs. **Phase I enzymes**, such as those belonging to the cytochrome P450 family, are very important for the metabolizing of drugs. **CYP2D6** is not a major CYP enzyme in terms of quantity in the liver, but it metabolizes one quarter of all drugs, and many of the ones used in hypertension. Poor metabolizers display exaggerated response and may be at greater risk for toxicity if a drug is principally metabolized by CYP2D6. In contrast, ultra-rapid metabolizers may not achieve therapeutically active plasma concentration for certain drugs. Genotyping an individual by Polymerase Chain Reaction (PCR) methods can be used to predict the metabolizer status, and specific DNA chips are now on the market, such as AmpliChip®, marketed by Roche Diagnostics. **CYP3A** enzymes metabolize circa 50% of all drugs. As such, they are often implicated in ADRs and drug-drug interactions;

• **Phase II enzymes**, such as N-acetyltransferase 2 (NAT-2) and UDP-glucuronosyl-transferases (UGTs), are not considered to have a great impact in the metabolizing of antihypertensive drugs;

• **Phase III enzymes** (drug transporters), have important roles as they determine drug disposition, intestinal absorption and renal elimination. There are five classes of uptake carrier systems (organic anion transporters –OATPs; organic cation transporters – OCTs; dipeptide transporters – PEPTS; nucleoside transporters – CNTs; monocarboxylate transporters – MCTs) and the ATP-binding transporter related to the efflux of drugs and metabolites (ABC). Another major drug transporter across renal and intestinal cells is P-glycoprotein (P-gp). Verapamil is an antihypertensive drug that is a substract to both CYP3A and P-gp.

Table 6 - Examples of anti-hypertensive drugs affected by polymorphisms of the CYP450 family enzymes and transporters

Phase I enzymes and transporters implicated in anti-hypertensive drug metabolism (examples)		
Substrates	**Enzyme (CYP450 family)**	**Transporters**
Losartan	3A, 2D9	ABCB1
Carvedilol	3A, 2D6	ABCB1
Verapamil	1A2, 3A, 2C8	ABCB1, OCT1
Enalapril	3A	OATP, PEPT
Captopril	2D6	OATP, PEPT
Diltiazem	3A, 2C9, 2D6	ABCB1

Pharmacodynamic related genes:

Equally important is the evaluation of variations in gene sequence of pharmacological targets. There are several pharmacological targets, including receptors, enzymes, ion channels, lipoproteins and signal transduction pathways. They are localized or expressed differently in tissues and cells. These targets exhibit genetic variability that can alter the binding affinity of a drug or its metabolites, and thus modulate drug response. For antihypertensive drugs, the main described polymorphisms influencing pharmacodynamics are:

i. Angiotensinogen (AGT);
ii. Angiotensin II receptor type 1 (AGTR1);
iii. Angiotensin-converting enzyme (ACE);
iv. α-adducin (ADD1);
v. Bradikinin receptor B2 (BDKRB2).

Table 7 - Examples of anti-hypertensive drugs affected by gene polymorphisms that influence the pharmacodinamic phase of drug actions

Polymorphic genes influencing pharmacodynamics actions of anti-hypertensive drugs		
Gene	**Example**	**Clinical consequences**
A-adducin	Hydrochlorothiazide (HCTZ)	Greater reduction in blood pressure to HCTZ treatment
ACE	Enalapril	Greater and longer drug response
AGT	Several drugs	Reduction of blood pressure and decrease in left ventricular mass with anti-hypertensive treatment
AGTR1	ARB	Increase arterial responsiveness to angiotensin II in ischaemic heart disease and increased aortic stiffness in hypertension
BDKRB2	ACEI	ACEI related cough

In Figure 16 is depicted the pathway of the drugs acting in the RAAS as well as the genes involved in the PD of the same drugs.

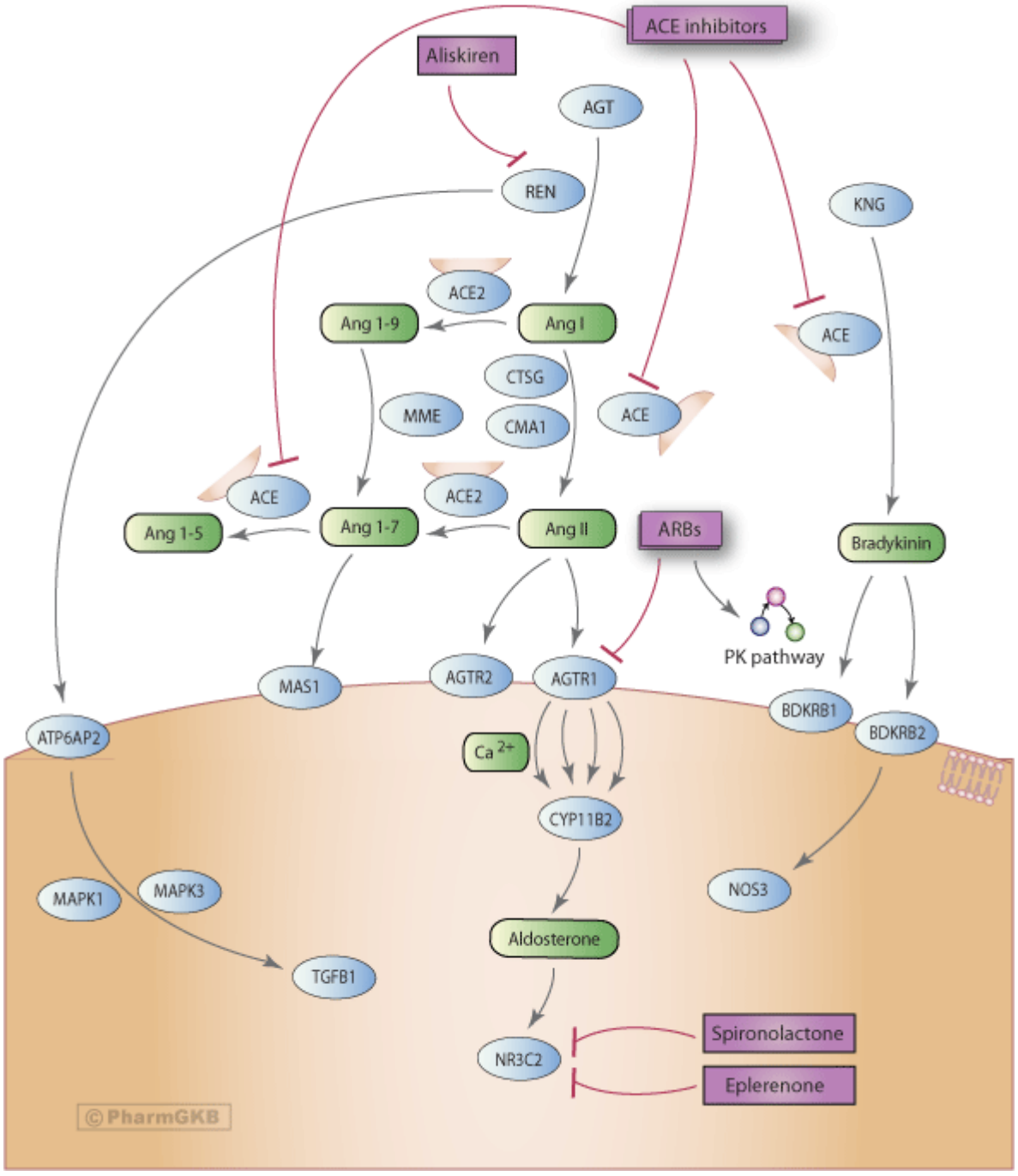

Figure 16 - Genes involved in the pharmacodynamics of the drugs that act on the renin-angiotensin-aldosterone system. The renin-angiotensin-aldosterone system is central to the control of blood pressure and the target of several types of anti-hypertensive drugs. This pathway depicts a simplified representation of the pharmacodynamics of RAAS-acting drugs including candidate genes for the pharmacogenomics of ACE inhibitors, angiotensin receptor blockers, renin inhibitor aliskiren and aldosterone receptor antagonists. The core of this pathway involves the conversion of angiotensinogen to angiotensin I (Ang I) by renin, its subsequent conversion to angiotensin II (Ang II) by angiotensin converting enzyme. Ang II activates the angiotensin II receptor type 1 to induce aldosterone synthesis, increasing water and salt resorption and potassium excretion in the kidney and increasing blood pressure. The key candidate genes involved in the core pathway are angiotensiogen, AGT; renin, REN; angiotensin converting enzyme, ACE; and angiotensin II receptor type 1, AGTR1. (Adapted from http://www.pharmgkb.org/pathway with permission given by PharmGKB and Stanford University).

There is often a family history of high blood pressure in hypertensive patients, suggesting that inheritance contributes to the pathogenesis of this disorder. Essential hypertension is a highly heterogeneous disorder, which points to a multi-factorial and polygenic etiology. Variants in some genes might render an individual sensitive to a given factor in the environment. On the other hand, the patient´s genetic predisposition might influence drug-metabolizing enzymes and this in turn might affect both efficacy and adverse effects of antihypertensive therapy [(2)].

4.1 Anti-hypertensive Drugs and Pharmacogenomic Studies

Renin-Angiotensin-Aldosterone System

Genetic variations corresponding to this system have been shown to be associated with a tendency to high blood pressure:

- ***ACE inhibitors***: the ACE insertion/deletion polymorphism is one of the most known polymorphism of the RAAS which is linked to hypertension. The deletion allele of the polymorphism is strongly associated with an increased level of circulating ACE, which can alter the response to ACEI;

- ***ARBs***: angiotensin receptor blockers provide a complete and specific suppression of the RAAS. They decrease blood pressure by blocking the binding of Ang II to the angiotensin type 1 (AT1) receptor. Also, ARB administration indirectly activates the AT2 receptor by blocking feedback inhibition of renin release and shunting the angiotensin II generated from AT1 and AT2. The A1166C polymorphism of the AT1 receptor gene was studied in relation to the response to losartan and was found that patients with the CC genotype were less responsive than with the AA genotype [(3)]. This is an example, if this marker reveals to be strong, where a pharmacogenomic test can be used to predict the response to losartan;

- ***Renin Inhibitors***: overexpression of renin predisposes individuals to develop hypertension. Aliskiren is presently, the only available drug that belongs to this group. Although there are some studies, they are not yet predictive for the use of this drug.

Beta-Blockers

Beta-blockers are widely used in hypertensive patients. Two major polymorphisms encoded by the beta-1 adrenergic receptor gene – ADRB1 - are commonly used in both hypertension and heart failure pharmacogenetics studies of beta-blockers. These

polymorphisms result in an amino acid substitution at codon 389 (Arg389Gly, C1165Gpolymorphism) that codes for the intracellular part of the receptor and at codon 49 (Ser49Gly, A145G polymorphism) that codes for the extracellular part of the receptor. Based on the functional data of these SNPs, they have been extensively studied for associations with response to beta-blockers, with the hypotheses that the more responsive Ser49 and Arg389 forms would be the ones with the greatest β-blocker efficacy [4].

Diuretics

The C825T polymorphism of the G-protein beta-3-gene (GNB3) appears to predict patient response to thiazide diuretics, while CA repeat length of the 11-beta- hydroxysteroid dehydrogenase type 2 (HSD11B2) gene has been strongly associated with the blood pressure response to HCTZ [1].

4.2 Future directions

Despite all the studies performed regarding this subject – Pharmacogenomics and Hypertension - a strong relation between genomic variance and drug response is not yet fully established.

A search performed in the website of the National Centre of Biotechnology Investigation, under the PubMed link (http://www.ncbi.nlm.nih.gov/pubmed?term=pharmacogenomics), made in the 15th of December 2012, showed that there were published 1,165 papers under the search term "pharmacogenomics" in the current year (321 reviews), and 16,435 papers under the search term "hypertension". When the search was performed under the search terms "pharmacogenomics and hypertension" only 25 were found, two of which were related to pulmonary hypertension. Under the search term "pharmacogenomics and cancer", in the current year, 225 papers were published.

One of the most helpful PGx databases, as considered by the American Pharmacist Association, is available at http://www.pharmgkb.com. In this web site, which is presented as "a comprehensive resource that curates knowledge about the impact of genetic variation on drug response for clinicians and researchers", a specific link can be found to well-known pharmacogenomic associations. Given our study subject – pharmacogenomics of

antihypertensive drugs – it could only be found direct therapeutic dose recommendations for one drug: metoprolol (a beta-blocker).

Also, in 2011, the National Heart, Lung, and Blood Institute Working Group (USA), issued a report regarding cardiovascular pharmacogenomics current status and future directions, where three major emerging PGx applications were reviewed: anticoagulation (warfarin), antiplatelet therapy (clopidogrel), and lipid-lowering therapy (statins) [(5)]. Pharmacogenomic studies of anti-hypertensive drugs were not considered a "pressing clinical need".

Nevertheless "genetic determinants of blood pressure and long-term outcomes in hypertensive patients are being identified, and their role in choosing among therapies is under active investigation" [(6)].

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5. Pharmacogenomics in the Community Pharmacy Practice

The Community Pharmacy and its community pharmacists are important pillars in the delivery of health products to the population: drug products, technical aids, point-of-care tests or pharmaceutical care plans are some of the services already available at any pharmacy. Being able to provide PGx testing in a community pharmacy setting gives the pharmacist yet another opportunity to help individualize drug therapy, maximising efficacy (the right drug and the right dose) and minimising toxicity based on patients' genetic data. Pharmacogenomic tests along with other point-of-care tests can increase access to information that can be used to guide and monitor drug therapy.

The community pharmacy is equipped with both professionals and facilities that can easily integrate this practice in its many provided services and products.

The community pharmacist has a broad knowledge in pharmacology that allows him/her to easily acquire the skills necessary to implement a PGx service in the community pharmacy practice. Nevertheless, it is compulsory that for being able to provide this service, the pharmacist will have to acquire deep knowledge in PGx, as it integrates not only specific technical aspects such as handling patient samples but also cognitive actions such as critical appraisal of evidence (need to be up-to date with current drug monitoring technics and scientific literature), decision-making, interpreting test results and providing counselling to patients. On the other hand, these latter activities are not strange to the pharmacist as they are already employed in several services provided.

The community pharmacy has evolved in its purpose, especially in the last two decades, from being where the "drug specialist" works and drugs are sold, to where patients can find professionals that are interested in their well-being, providing services that can answer specific questions and helping monitor the results in each counselling. This meant some changes, even in the physical structure of the community pharmacy, where now it can always be found a place where patient and pharmacist can talk in private and where an increasing number of services are performed, such as vaccination, drug administration and monitoring analysis. In fact, the community pharmacy is highly equipped to include PGx in its service portfolio.

5.1 Integrating PGx in the Community Pharmacy Practice

A community pharmacist has to able to improve drug efficacy and safety, by having more knowledge of the current drug monitoring technics, pharmacogenomic tests included.

Nowadays, pharmacovigilance plays an important part in providing information on drug safety, and it is common for a pharmacist to refer back to the prescribing physician any possible drug-drug interaction or side effect. Nevertheless, pharmacogenomic testing could avoid those unwanted effects.

Recently, a new website was launched, in Portugal, which enables healthcare professionals and members of the public to report any reaction to a drug: http://extranet.infarmed.pt/page.seram.frontoffice.seramhomepage.

Figure 17 - Portuguese website that enables reporting adverse drug reactions (http://extranet.infarmed.pt/page.seram.frontoffice.seramhomepage).

Not long ago, reporting a side-effect of a drug was considered to be the physician´s chore, but as the society is becoming more active, where every part involved in one given medical treatment has the right and duty of alerting to the consequences of such treatment, good or bad. Patients and all healthcare professionals should not only be proficient in choosing the right drug but also in avoiding the drugs side-effects, and implementing pharmacogenomics in the common practice is a clear path to follow.

Every health professional should be educated in genetics and pharmacogenomics sciences, not only those professionals who prescribe drugs, but also those who dispense and monitor them.

Graduated pharmacy studies should offer courses in such a subject and a post-graduation should be presented to those pharmacists who wish to deepen their knowledge in PGx.

Newly graduated pharmacists, although equipped with many knowledge involved in the understanding of PGx science, need to be in a continuous educational program to become proficient in this matter. This, then, must be the first step: **educating community pharmacists in PGx**.

However is not only the community pharmacist that needs to be educated in PGx: physicians and patients also need to know about this rapidly evolving science, especially in what it means to be tested for specific pharmacogenomic traits and how it will be helpful when establishing a drug therapy. And here, the community pharmacist may also play a predominant role: the pharmacist can be the conveyer of knowledge in PGx to the physician and patient by facilitating the access to PGx testing and assisting in physician and patient education.

But, being able to provide pharmacogenomics services in a community pharmacy poses some likely questions such as:

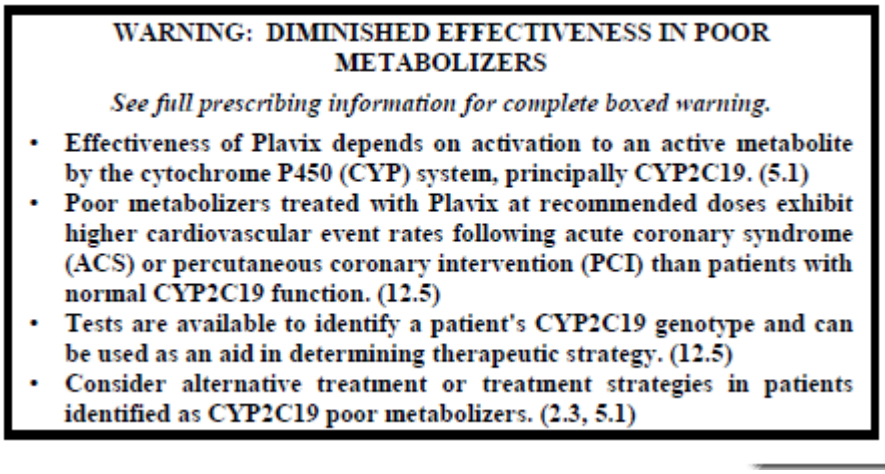
- What role must the pharmacist have in providing such services?;
- What may be the impact that these services on the practice of pharmacy?;
- What are pharmacogenomic services, and what activities must a pharmacist undertake to provide those services?;
- What is their clinical utility?;
- Are these tests cost-effective?

If there is sufficient interest and demand from patients, it is in the community pharmacy that these tests will have a major impact. Not only in acknowledging the possibility of taking these tests, but also in providing them to the patients, making sense of them and finally advising the patients accordingly.

This means, of course, that pharmacists would require training in the interpretation of test results, as well as dealing with ethical concerns and issues of patient confidentiality.

For a community pharmacist, it is important to know what it means to have a particular genomic alteration, but also to know what it implies for the patient: are there alternative drugs?, may the patient thrive without medication? The pharmacist has to be able to answer the patient's questions about the result of the test and know where to get as much information as possible.

Also, clear recommendations must be given from the drug producer or health authority, on what pharmacogenomic test has to be performed, in which circumstance, for which patient, regarding which health condition. As seen in Table 5, there are already drugs with PGx information in their labels. Some, but not all, of the labels include specific actions to be taken based on genetic information. An example of a box warning present in a drug label is depicted in Figure 18. Biomarkers may include gene variants, functional deficiencies, expression changes, chromosomal abnormalities, and others.



WARNING: DIMINISHED EFFECTIVENESS IN POOR METABOLIZERS

See full prescribing information for complete boxed warning.

- **Effectiveness of Plavix depends on activation to an active metabolite by the cytochrome P450 (CYP) system, principally CYP2C19. (5.1)**
- **Poor metabolizers treated with Plavix at recommended doses exhibit higher cardiovascular event rates following acute coronary syndrome (ACS) or percutaneous coronary intervention (PCI) than patients with normal CYP2C19 function. (12.5)**
- **Tests are available to identify a patient's CYP2C19 genotype and can be used as an aid in determining therapeutic strategy. (12.5)**
- **Consider alternative treatment or treatment strategies in patients identified as CYP2C19 poor metabolizers. (2.3, 5.1)**

Figure 18 - Excerpt of the Plavix® label (box warning), where is stated that CYP2C19 weak metabolizers need to titrate drug dosage and information about drug-drug interaction.

5.2 Proposed Action

The integration of PGx in the community pharmacy seems not to be difficult to achieve, as it means to provide an alternative or additional test that can be performed at the pharmacy, by the pharmacist, and which result can also be checked and acted upon in the same place.

Instead of doing the theoretical risk-assessment based on the current, and sometimes weak, knowledge of the patient health condition, the physician's diagnostic (if known) and the prescription available, the pharmacist can offer the patient an additional PGx test to confirm the relevance of the drugs prescribed and to which extent they may be able to cause ADRs or interact with other drugs used by the him/her.

Exactly, what kind of service is involved in pharmacogenomic testing?

Within the current pharmacy practice framework, pharmacogenomics advice can be given based on information from various sources and by the pharmacist at the point of dispensing or sale. The advisory role of the pharmacist, which includes informing patients about how to take their medications and how to avoid drug-drug and drug-diet interactions, can be expanded to include pharmacogenomics.

As for the providing of the tests, there should be no major difference from what is already done when glucose, cholesterol or blood pressure measurements are made in a pharmacy setting. The sample collection for a PGx test usually includes a mouth swab, a painless and non-invasive technic which allows the collection of cells from which is extracted genetic material needed to perform the test. In a first approach, the PGx test itself will not be performed at the pharmacy, pharmacists will only have to collect the sample and send it to a referenced laboratory.

Nevertheless, a pharmacist has to undertake some activities if he/she wants to offer pharmacogenomics services, such as being able to:

1. Establish a professional understanding with the patient's physician – for a PGx service to be successful, a trustful and professional relationship between pharmacist and physician must be established. Otherwise, the result of a PGx test may not lead to changes needed in drug therapy, as the patient may not feel safe if his/her physician does not take part in this decision;
2. Critically appraise evidence – not all patients need a PGx test. It is extremely important for a pharmacist to be able to determine the relevance of such test in patient care;
3. Make decisions and manage risk – once the result of a PGx is known, decisions have to be taken and the pharmacist has to be able to make recommendations based in those results, always bearing in mind the patient's safety;
4. Understand one's own limits – as a part of a team (physician-patient-pharmacist) the pharmacist must know that every member of the team must agree on the action to be taken and whether this service is of not helpful for the patient;
5. Obtain, handle and test patient samples;
6. Solve problems and keep appropriate records – it is of extreme importance to keep recorded all facts and figures involved in a given PGx test for every particular patient. This will back up actions taken and permit following reviews, if needed;

7. Develop diagnostic and disease monitoring skills, synthesize patient data and genetic data;

8. Communicate effectively and provide relevant counselling to both patient and physician - what is the value of testing, what does each result mean, what can be done upon getting the result, what's expected to happen after any action is taken;

9. Obtain informed consent – compulsory.

In Figure 19 is depicted a flowchart that illustrates the actions that can be taken when performing a PGx test in a community pharmacy. When filling a prescription or when asked by a patient, a PGx educated pharmacist should be able to identify any PGx relevance related to the drug(s) prescribed. If there is a PGx test available and it is found suitable and feasible for a particular patient, then information about it, such as how it works, what means to take a PGx test and what can be done upon getting the result, must be clearly presented to the patient. Once the patient decides to take the test, the physician must be informed, not only that there is a PGx available for his/her patient but also about the willingness of the patient to be tested. Before samples are collected an Informed Consent must be signed.

The sample collection is done, for most of the tests available, by a mouth swab, which is considered a non-invasive and a rapid bio-sample collection method. It is very easy to perform, with virtually no restrain. After the sample is collected it must be sent to a referenced laboratory where the PGx study will be performed. The pharmacist should then receive the results and its report as soon as possible.

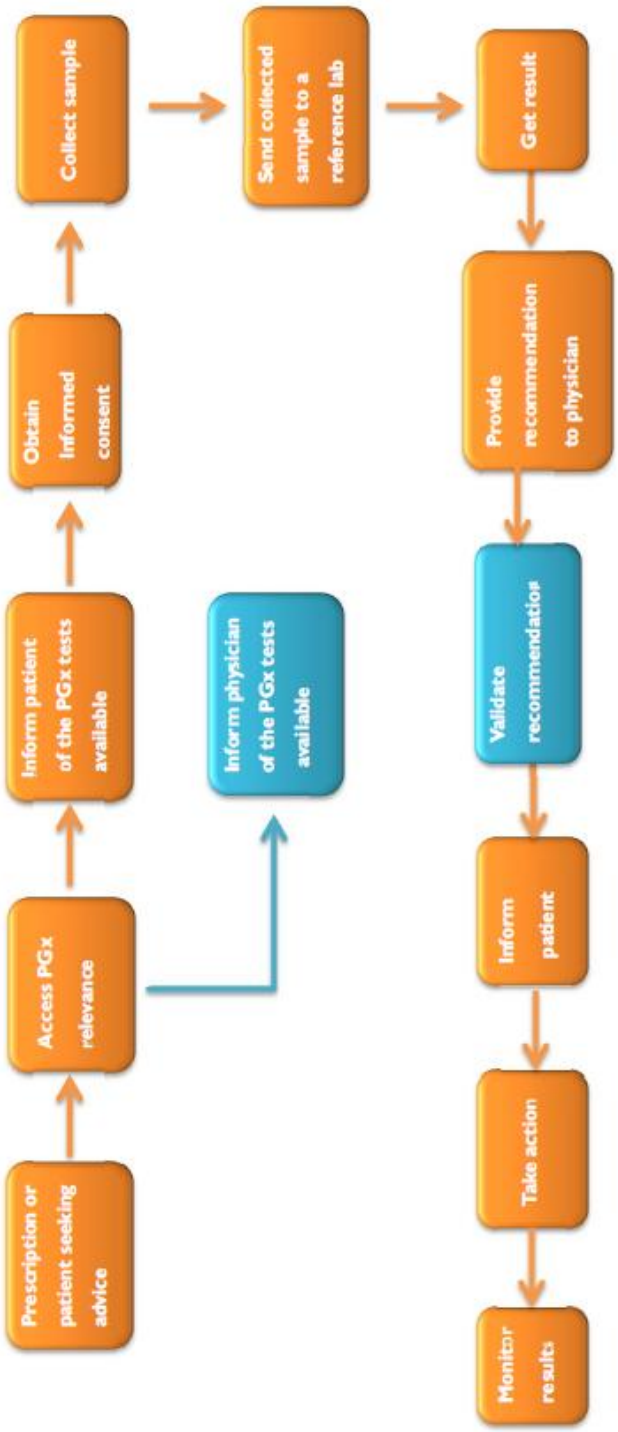


Figure 19 - PGx testing in a Community Pharmacy. To implement PGx testing in a community pharmacy the pharmacist has to undertake a number of specific activities that are depicted in the above workflow: he/she as to be able to recognise whether the patient has a condition where it is relevant to perform a PGx test. Informing the patient as to what a PGx test refers to (as well as informing the physician) is extremely important because this will lead the obtaining of an informed consent which allows the collection of the biological sample. The sample collected must then be sent to the reference laboratory, from where the results and report are sent back to the pharmacist. The pharmacist must then provide a recommendation based on the results and report to the physician. Finally the patient is informed, an action is taken and the pharmacist continuously monitors the results of the action performed. Blue boxes indicate the physician's activities, orange boxes indicate the pharmacist's activities.

Time is of essence when a therapy must be initiated as soon as possible or a change in therapy may bring major benefits for a given patient. Ideally, the exchange of information between pharmacist-lab-physician-patient should have little interference and it should be done effectively and rapidly. It will be most helpful to create a web based information system which enables this exchange of information to be clear, specific, patient and result oriented, that allows the recording of all results and actions taken, that can be a useful guide to the patient, but also easy to consult by the parties involved, and that makes it possible to look back on actions taken and better interpret what is involved in this kind of study. A PGx testing data flow diagram, which can be built as a web based platform, is proposed in Figure 20.

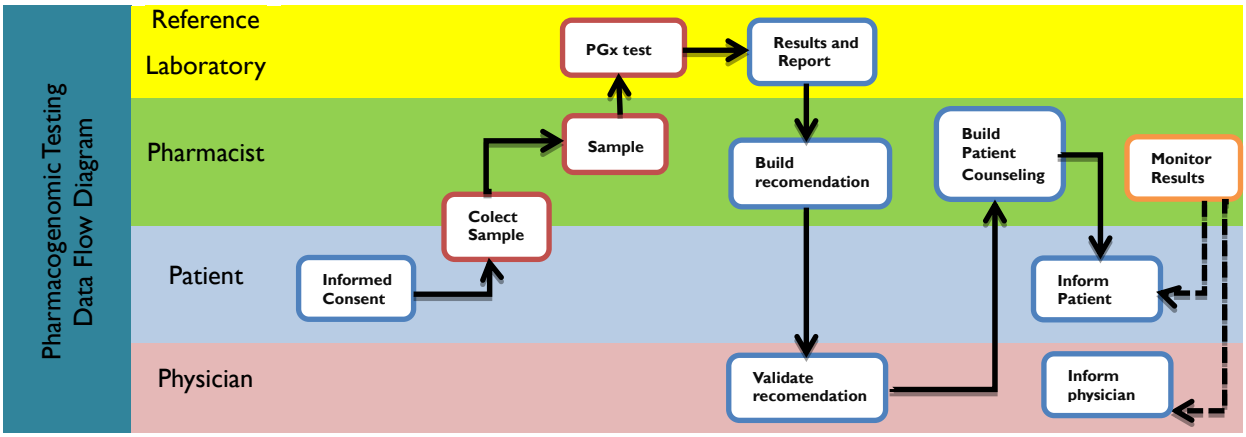


Figure 20 - Pharmacogenomic Testing Data Flow Diagram. Blue boxes indicate actions that can be performed in a web based information system, red boxes indicate actions performed by the indicated intervenient, which can be recorded in the information system but are performed outside, and the orange box indicates an activity that is performed only by the pharmacist, inside the information system, and that can report back to the patient and physician.

In this valuable exchange of information between pharmacist, laboratory, physician and patient, where no data must be lost or undervalued, it is highly important to count on a reliable information system. The development of an information system should be coded as a web based platform easily available to every part involved in a PGx assessment. Nevertheless, if or when this platform is not available, the use of other means of communication such as fax, telephone, mail or even face-to-face consultations may be performed.

When a pharmacist receives the report of a PGx test he/she must build a recommendation based on the results with the help of algorithms and on up-to-date scientific literature. Only then must the pharmacist send the results and report of the PGx test, along with the recommended actions to the physician. The physician must, then, validate

the proposed recommendation, whether accepting or rejecting it, or even adding its own recommendation. Finally, the patient must be informed.

But the role of the pharmacist must not end here. The monitoring of the results of the actions taken (suspend, switch or titrate medication) can and must be performed by the community pharmacist.

As was stated before, for anti-hypertensive drugs, there are few labelled recommendations, and of the ones presented none is stringent to the performance of a PGx test. Nevertheless, as this is a therapeutic class of drugs that is prescribed based a trial-and-error practice, the use of these tests may be of great help in the near future. Also, it is a novel opportunity for community pharmacist to gain greater recognition as members of the primary health care team.

References:

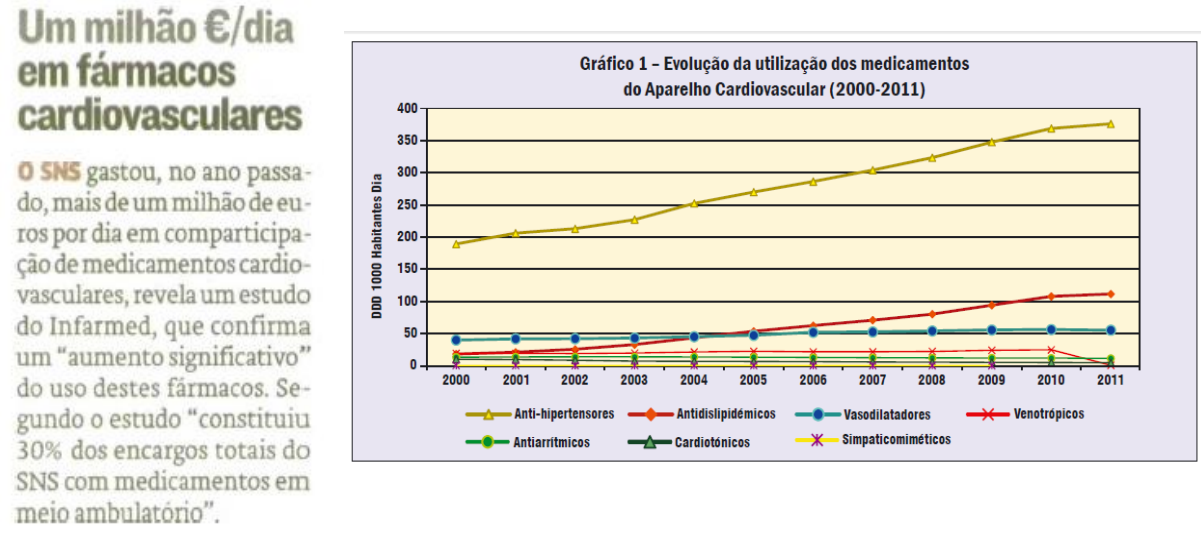
1. NEWTON Robert et al. - **How will pharmacogenetics impact on pharmacy practice? Pharmacist´s view and educational priorities.** NHS, National Genetics Education and Development Centre, London, November 2007.

2. GRECO J. - **Pharmacogenomics in Community Pharmacy Practice: A Look into the Future of Personalized Medicine.** *J* Am Pharm Assoc. 2011;51:189-193.

6. Conclusion

Pharmacogenomics is a new science with many obstacles yet to overcome. Lack of comparative and cost-effectiveness data (no clinical trials or studies were found that reflected cost-effectiveness data of a PGx test, although it is commonly stated that it may bring savings to both patient and reimbursement system) and the need for education and clinical practice guidelines are probably the most pungent issues to assess. In the specific case of PGx in hypertension treatment, the search of solid biomarkers that can help make a decision for treatment is far from being completed. But, it is believed that the implementation of the PGx testing should be at pace with science and not be far behind. Although PGx testing is taking its first steps, several healthcare communities are starting to implement these tests: in Australia, there are already timelines for the implementation of PGx in the community pharmacies, being the short-term implementations set prior to 30 June 2015, medium-term between 30 June 2015 and 30 June 2020, and longer-term after 1 July 2020 [1].

Applying PGx tests to patients under anti-hypertensive treatment is a challenge. Recently, in November 2012, the *Jornal de Notícias*, reported that, according to the Infarmed (Portuguese Health Authority), the Portuguese public health system spent over one million euros per day in cardiovascular drugs reimbursement (Figure 21).



Um milhão €/dia em fármacos cardiovasculares

O SNS gastou, no ano passado, mais de um milhão de euros por dia em comparticipação de medicamentos cardiovasculares, revela um estudo do Infarmed, que confirma um "aumento significativo" do uso destes fármacos. Segundo o estudo "constituiu 30% dos encargos totais do SNS com medicamentos em meio ambulatório".

Figure 21 - Excerpt of Jornal de Notícias, 09 November 2012 (on the left): "The SNS (Portuguese public health system) spent, last year, over a million euros per day in the reimbursement of cardiovascular drugs according to a study performed

by Infarmed, which confirms a significant raise in the use of these drugs. According to this study, cardiovascular drugs account for 30% of total SNS expenditure in ambulatory". On the right side of the figure is a chart, published in the Infarmed periodic publication – Infarmed Notícias – in November 2012, which shows the evolution of the use of cardiovascular drugs over the last decade. The dark yellow line represents the anti-hypertensive drugs, which account for the most used drugs in the treatment of cardiovascular diseases. The article news from Jornal de Notícias was written based on this article of Infarmed Notícias. (Available from: http://www.infarmed.pt/portal/page/portal/INFARMED/PUBLICACOES/INFARMED_NOTICIAS/infarmed%20not%EDcias%20-%20N.%BA%2044%20-novembro%202012%20-%20internet.pdf).

These numbers clearly show how much the cardiovascular drugs weight in the public expenditure. On the other hand, they do not reflect the money spent with ADRs, drug-drug interactions or redundant pharmacotherapy that may occur. This can mean that PGx tests, although they may not be inexpensive (at least not until they are routinely used), can help bring these numbers down, for the benefit of patients and health systems. With so much money spent in the treatment of hypertensive patients, the investments made to better guide drug treatment should not be underestimated.

Pharmacogenomics has the potential to substantially change health practice and the expectation of stakeholders such as health professionals and governments, and reinforce the position of the community pharmacist as a healthcare professional. Over the last five years, especially since the price of drugs plummeted, much due to the implementation of generic drugs in Portugal, and due to the world economic crisis, the community pharmacy has met serious financial difficulties. In July 2012, a study performed by *Nova School of Business & Economics*, showed that more than 1100 pharmacies, out of a total of 2900, were in an "unbearable situation" [(2)]. The implementation of PGx testing could also help the community pharmacists to find new fields of actions to better thrive.

The implementation of PGx testing is not only concern of the pharmacist, patient and physician. Other parties, such as consumer organizations, disease management organizations, government and regulatory bodies, pathology organizations, pharmacy organizations, pharmacy software developers/vendors, product manufacturers, insurance companies, reimbursement system and any health professional bodies can and will take active part in the development and implementation of these tests. It is high time to incorporate PGx testing into the everyday practice in clinical and pharmacy settings.

References:

1. **Community Pharmacy Roadmap Program Development Template**. The Pharmacy Guild of Australia. [Accessed in 12 July 2012]. Available from: http://www.guild.org.au/iwov-resources/documents/The_Guild/PDFs/News%20and%20Events/Publications/The%20Roadmap/Roadmap%20-%20Pharmacogenomics.pdf.

2. **Mais de 1100 farmácias em dificuldades com fornecimentos suspensos**. Público, 12 July 2012. [Accessed in 12 July 2012]. Available from http://www.jn.pt/PaginaInicial/Sociedade/Saude/Interior.aspx?content_id=2662092.