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# Individual response to drug therapy: bases and study approaches

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**ABSTRACT**

Genomic variation largely explains the differences in an individual's response to drug treatments. A field of genomic medicine focuses on the identification of genetic polymorphisms and gene mutations involved in the development and progression of disease. Another part focuses on the development of genetic tests to accompany medical prescriptions, to predict how certain patients respond to therapy with a given pharmacological agent. The field of predicting responses to drugs has different strategies and methods, among which we find: the use of liver microsomes, cell models, monitoring of probe drugs, assays with recombinant proteins and recently the use of microarray platforms or DNAchips.

**Key words.** Drugs. Human genome. Individualized medicine.

***Respuesta individual a terapias farmacológicas: Bases y enfoques de estudio***

***RESUMEN***

*La variación genómica explica las diferencias de los individuos para responder a los tratamientos farmacológicos. El campo de la predicción de las respuestas a fármacos dispone de diversas estrategias y métodos, entre ellos: microsomas hepáticos, modelos celulares, monitoreo de fármacos sonda, ensayos con proteínas recombinantes y recientemente el uso de dispositivos diagnósticos de la era genómica, como microarreglos o DNAchips. Un área de la medicina genómica se enfoca en la identificación de polimorfismos genéticos y mutaciones en genes que intervienen en el desarrollo y progresión de las enfermedades. Otra parte se concentra en el desarrollo de pruebas genéticas de acompañamiento a las prescripciones médicas para predecir cómo ciertos pacientes responden a las terapias con un agente farmacológico dado.*

***Palabras clave.*** *Fármacos. Genoma humano. Medicina individualizada.*

## GENETICS IN DRUG METABOLISM

There are interindividual differences in drug metabolism that are influenced by factors such as age, sex, diseases, the environment, diet and drug interactions, as well as by each person's genetic profile (genetic variation).[1] Although pharmaceutical companies prove the efficacy and safety of drugs before placing them on the market, there is interindividual variability in drug metabolism and the response to these, which is a consequence of the interaction of the aforementioned factors. The discrepancy between plasma concentrations in two physiologically similar subjects who were administered the same dose of a drug, can be > 1000-fold.[2] Ingelman-Sundberg stated that "genetic factors can account for 20% and 40% of these interindividual differences in metabolism and drug response; however, for certain drugs or classes of drugs, genetic factors are the most important for the outcome of drug therapy."[2]

The scientific area, which began to explain the role of genetic variation in drug metabolism, had its dawn formally at the end of the decade of 1950.[3] In 1959 Friedrich Vogel, christened this area of scientific research as pharmacogenetics. This field had a slow growth over the next few years because in only a few drugs, the response or the presence of an adverse drug reaction (ADR), was associated to a single gen.[4]

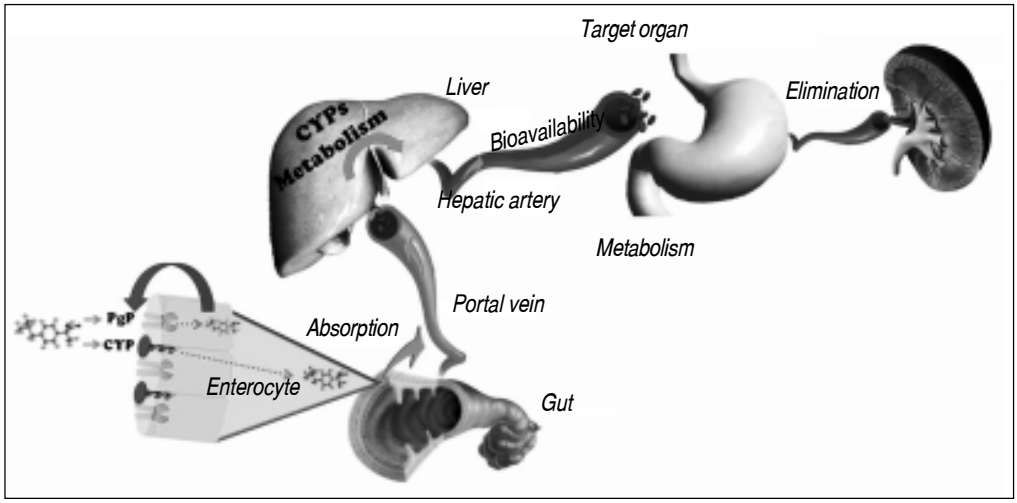


*Figure 1. Drug metabolism. After administration, drugs: 1) Interact and are absorbed by enterocytes. 2) Pass into the bloodstream via the hepatic portal route. 3) Are metabolized by the liver enzyme cytochrome P450 family. 4) Are made bioavailable through the hepatic artery. 5) Exert their effect on the target organ. 6) Are modified and then eliminated by the kidneys.*

In 1997, as technology progressed, analyses, using a genomic mapping system (high density nucleotide arrays), of the genetic variability of pathways related to drug metabolism were proposed, parallel with that of genes associated with diseases, which was dubbed as pharmacogenomics.[5]

The terms pharmacogenetics and pharmacogenomics tend to get confused and although both study the variation in metabolism genes and drug response, they are different from each other. Strictly speaking, pharmacogenetics refers to the identification of variants, gene after gene, which affects the metabolism of drugs. On the other hand, pharmacogenomics refers to the simultaneous screening of the entire genome with genomics techniques, such as DNAchips, for variability in genes that are involved and interact in the metabolism, transport and response to drugs, to determine their therapeutic efficacy or safety.[6]

Even before the official conclusion in 2003 of the Human Genome Project (HGP), an exponential progress in science has been made. As a result, pharmacogenomic tests have been developed, such as new DNAchips that predict the response to pharmacologic treatments.[4] Pharmacogenomics is particularly revolutionizing the way a drug is prescribed, based on the revelation, from the genome, of each individual's ability to effectively or adversely (ADRs) respond to drugs (individualized medicine).[7]

Having an overview of genetic factors, current methods and strategies for the identification of these will allow a better understanding of the scope of pharmacogenomics in the prediction of therapeutic response.

## IMPACT OF THE HGP

With the fervent belief that a better understanding of the human genome could enhance unimaginable levels of biomedical research, in 1988 the United States government entrusted the Department of Energy (DOE), one of the most ambitious projects of scientific research: the Human Genome Project or HGP.[8] This great work was carried out by the DOE in collaboration with the National Institutes of Health (NIH) with the participation of other countries, becoming an international project (International Human Genome Sequencing Consortium or IHGSC). It formally began in 1990 with the primary purpose of knowing the complete human genome sequence so we can understand the evolution experienced by humans, know the number of genes that make up their genome, identify genomic variations, determine the genetic factors underlying diseases and how they interact with the environment in causing illneses.[9]

Due to the unusual development of sequencing equipment and the incorporation of robotics and computer control into most of the processes involved in the management and analysis of genomic libraries, the draft human genome sequence was completed in the year 2000,[10] three years ahead of schedule.[11] In April 2003, on the fiftieth anniversary of the publication of the structure of DNA, discovered by James Watson and Francis Crick, the project was concluded.

As a side effect of this project, biotechnology emerged, making open access databases available, such as the National Center for Biotechnology Information (NCBI: www.ncbi.nlm.nhi.gov). These

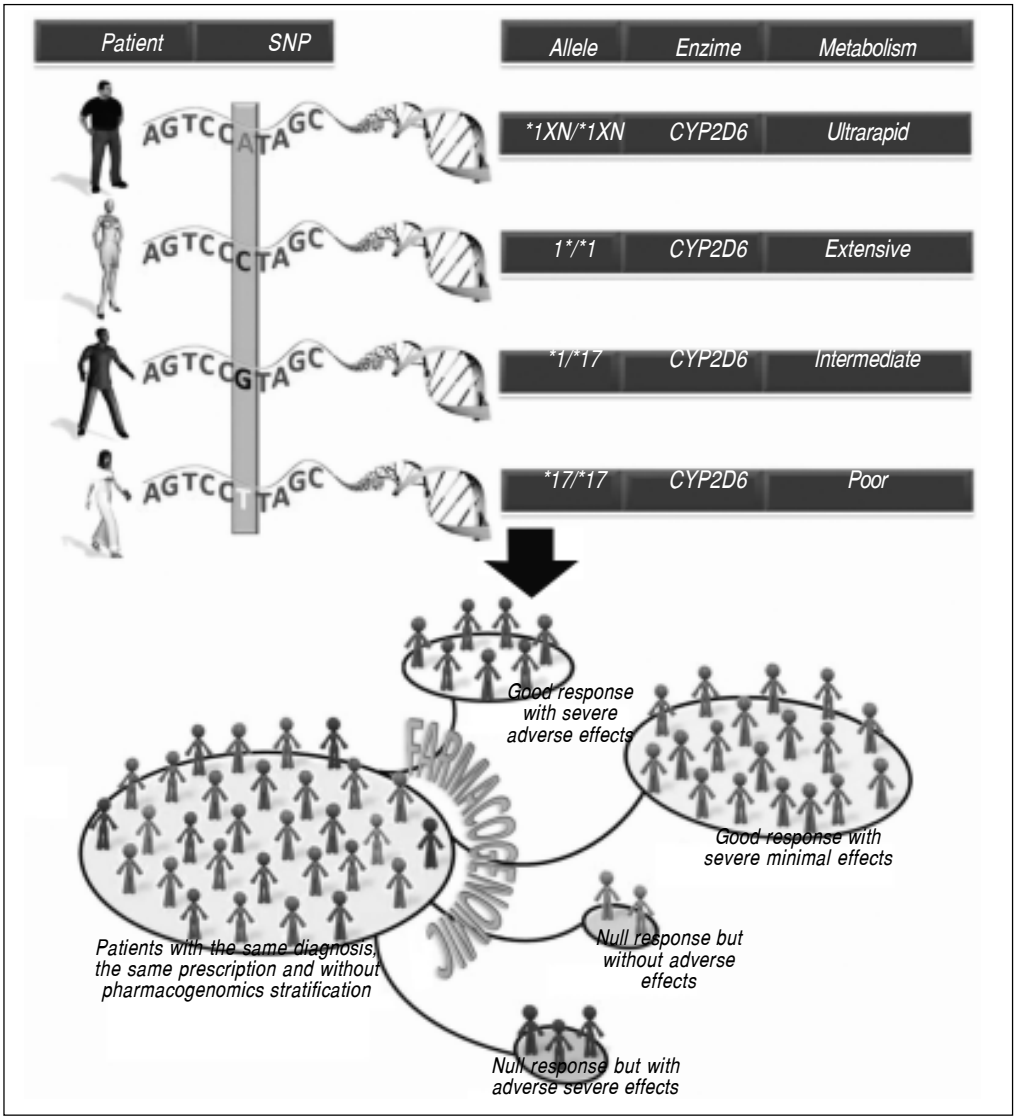


*Figure 2. Genetic Variability-Phenotype-Pharmacogenomics. **A.** The phenotype (metabolizer type) is linked to genotypic variability of individuals. Different alleles have been identified, distinguished by single nucleotide polymorphisms or SNPs in the same gene in different ways that affect drug metabolism. **B.** Using pharmacogenomic tools to track these SNPs, those who respond, those who do not and those who will present adverse effects can be distinguished from a group of people with the same diagnosis, prescription, and drug treatment. Modified from Kudzma,* et al.[54]

tools make information on genomic sequences of different species, among these humans, as well as information about inherited diseases and literature references, available to the scientific community. Thanks to this techno-scientific progress, computer programs that collect and monitor the activity of hundreds of genes simultaneously have developed.

The use of these tools has accelerated the discovery of genetic variations that predispose to diseases and others that can affect the response to pharmacologic treatments, enabling the development of new methods for predicting such risks.[10]

## AN "X-ray" OF GENOMIC VARIATION

With the draft sequence published in 2001 by the groups led by Venter and Collins, the objectives set by the CISGH were accomplished. Preliminary analysis of the sequence revealed that our genome contains about 3 billion nucleotide base pairs and it consists of approximately 23,000 genes, whose exons represent only 2% of the total sequence. The human genome is similar in the entire human population by 99.9% and the remaining 0.1% consists of the differences (genetic variation) existing between one individual and another.[8-10]

Genetic variation can be found as variations in the rate of single nucleotide polymorphisms or SNPs (Single Nucleotide Polymorphisms) and non SNPs, which are better known as structural variations (SVs). There are approximately 3 million and 400,000 of these kinds of variations, respectively, along the genoma.[9,12] Structural variations consist of small insertions and deletions (< 10 bp) or

indels, copy number variations or CNVs, and inversions.[12]

In recent comparative analyses, Pang and colleagues in 2010 report that "genetic variation is 1.2%, taking 0.8% small insertions and deletions/CNVs, 0.1% SNPs and about 0.3% inversions."[12] This amount of variation can increase when the 1,000 genome project is completed, begun in the year 2008, a work that is perhaps the most important with regard to the analysis of genetic variation, with which the CISGH aims to examine variations in greater detail throughout the genome (SNPs, structural variations and haplotypes). To achieve this, we are analyzing genomes of families, as well as unrelated individuals from around the world, are being analyzed. The primary objective is to further accelerate, investigation of biomedically relevant genetic factors in order to achieve a better understanding of how these affect health and the individual response to drugs.[13]

## VARIATION IN THE COMPOSITION OF THE GENOME

In the last decade researchers have focused on the study of genetic variation (polymorphisms). Polymorphisms are mutations that are found in at least 1% of the population, whereas mutations that occur in a smaller proportion are considered variants.[14]

For a better understanding of how genetic variation explains phenotypic differences, we must define the following terms:

In the case of non SNPs polymorphisms or structural variations, these are less frequent in the genome when compared with those of a single nucleotide, but with a greater repercussion.[15]

- **Indels (insertions and/or deletions).** They occur when one or more nucleotide bases are added or deleted (suppressed) in a region of the genome, these can vary in length from one to fifty base pairs. To mention one example, if these indels occur in coding regions they can cause aberrant non-functional proteins.[14]
- **Tandem repeats.** Madsen, *et al.* define these polymorphisms as those "1) that have a length of at least 9 bp, 2) if the repeat unit, e.g., AT, is repeated at least three times: 'ATATATATAT' and 3) if only a few base pairs in the segment are not equal to the repeat unit." This kind of variation can cause diseases such as Fragile X syndrome.[15]
- **Frameshift mutations.** When there are indels that are not multiple of three (characteristic of the genetic code), they cause a shift of the reading frame of the gene in question and the appearance of stop codons, therefore this produces a truncated protein that is affected in its function.[14]

  In the case of SNPs, which are the most abundant form of variation in the genome, the percentage reported by Pang, *et al.*, accounts for about 11 million SNPs, with these being more frequent in noncoding regions, with an estimate of one SNP for every 300 to 500 bp. SNPs are frequently found in introns or intergenic regions and are often called silent; however, in these regions regulatory elements that would be affected by the presence of a polymorphism can be found. The less common SNPs are located in coding regions at a rate of one per 1,000 bp that can be synonymous or non-synonymous depending on whether the nucleotide base change affects an amino acid exchange at the time of translation.[16]
- **Active site mutations.** When these mutations occur they can cause an amino acid substitution in the polypeptide chain that makes up the protein, which, although it may seem insignificant, can change the conformation of the protein and thus its affinity for the substrate or a ligand.[14]
- **Changes in messenger RNA processing or "splicing".** This type of mutation usually occurs in exon or intron regions near exon-intron junction sites, which is where the regulatory sequences of the removal of introns can be found. This may lead to alternative splicing or overlap, so that the mRNA ends up coding for a different polypeptide.[14]

Currently, the National Center for Biotechnology and Informatics or NCBI database has an inventory of more than 11 million SNPs and 3 million small indels.[12,13] The genetic variation that has received most attention from researchers, are the SNPs. These, because of their versatility, are used in association studies as markers to investigate which variants are associated with susceptibility to disease.[13]

## AMOUNT OF GENOME VARIATION

When speaking of amount of genome variation, we refer to the variation in the number of copies or CNVs. The CNV is defined as a fragment of genetic material or DNA (from one kilobase to seve-

ral megabases in length), which is present in a variable number of copies (by deletion or duplication), when compared against the genome of reference.[17] This type of structural variation is distributed throughout the genome's length and varies from population to population. The presence of discrepancies in the number of copies, has also been associated with complex diseases, such as susceptibility to HIV infection, autoimmune diseases, tumors, psychiatric disorders, mental retardation and autism.[18] Since the sections of DNA that are suppressed or duplicated reach great lengths, these segments can accommodate complete genes. This type of genetic variation is crucial in drug metabolism, where having multiple copies of a functional gene whose enzyme product is responsible for metabolizing a given drug, causes an increase in its clearance or removal, leading to a lack of efficacy at a standard dose. Alternatively, the lack of a copy of the gene due to deletion can result in a decreased or poor metabolism and consequently, in the accumulation of the drug, which causes the patient to have an ADR. In the opposite case where the drug is actually a pro-drug that requires its metabolism to lead to its active ingredient, the beneficiaries of its efficacy are rapid metabolizers and those who do not benefit are poor or slow metabolizers.[1]

## VARIATION IN DRUG METABOLISM

Most drugs are particularly lipophilic and therefore hydrophobic. This physicochemical property allows them to easily cross cell membranes, to their final destination (site of drug action) (Figure 1). However, because the human body is approximately 80% water, the same hydrophobic character of drugs interferes with its elimination from the body. As they accumulate and exceed their maximum safe concentration in blood (supratherapeutic level), they produce drug toxicity. To cope with this risk, our body has a repertoire of enzymes capable of biotransforming

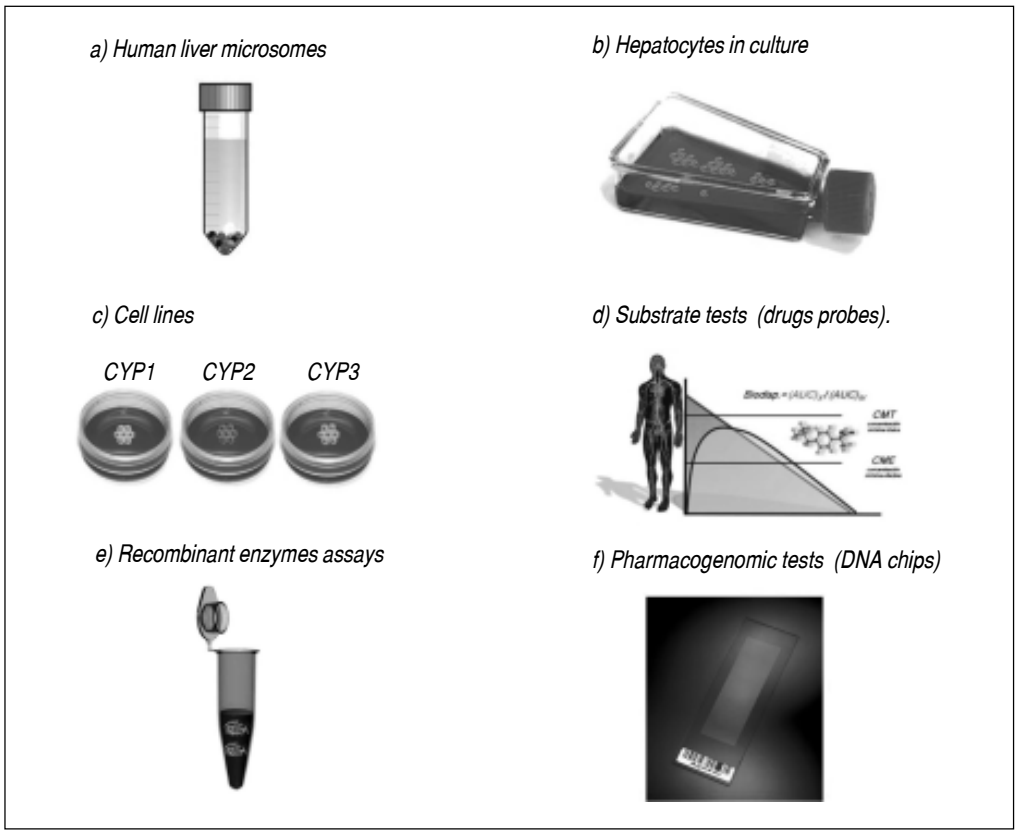


*Figure 3. Evaluation methods for Cytochrome P450 and its role in drug metabolism. An overview of the different methods of assessing drug metabolism is presented, with DNAChips being the fastest, the most reproducible, and better for simultaneously determining a large number of SNPs and mutations in all genes involved in drug metabolism. a) Liver microsomes assays are very versatile; however, they do not analyze genotype-dependent metabolism. b) Hepatocytes in culture have a good correlation* in vitro-in vivo*, but their availability is limited and they have technical requirements. c) Cell lines are an option for long-term trials, but it is difficult to extrapolate the results and they do not reproduce the physiological conditions of enzymes well. d) With substrate tests each subject can be fenotyped, but it is expensive and certain requirements must be met that make them less reproducible. e) Recombinant enzyme assays are an excellent option if you want to study genotype-dependent metabolism, since they have a good* in vitro-in vivo *correlation. However, these do not cover conjugating enzymes. f) Pharmacogenomic tests provide a good alternative to predict drug metabolism. They are fast and evaluate a large number of genes involved in drug metabolism. Modified from Donato,* et al.[40]

drugs.[19] The superfamily of isoenzymes of the hepatic cytochromes P450 or better known as CYP450, are responsible for this task. The metabolism of drugs is mainly composed of two phases:

- **Phase I.** Metabolism (oxidation-reduction reactions) that convert the metabolite to a more active or more soluble form.
- **Phase II.** Metabolism (conjugation reactions) that primarily converted hydrophobic molecules to more hydrophilic structures to facilitate their excretion. CYP450 through oxidative metabolism, convert about 80% of clinically used drugs and about 50% of eliminated drugs.

Genetic polymorphisms in the coding sequences of these enzymes, determine the response that an individual may have to a drug, which can be canceled, reduced, normal or even increased.[20,21]

## Genetic variation reflected in liver enzymes

In a study of comparative genomics between different species, Nelson, *et al.,* in 2004 identified 57 genes that code for different active isoforms of CYP450 enzymes, and a similar number of pseudogenes (http://drnelson.utmem.edu/CytochromeP450. html). The isoenzymes of the CYP450 family are involved in the metabolism of endogenous substances, for example prostaglandins, as well as those exogenous to the human body or xenobiotics, among which the most notable are food and drugs. Different types of genetic variation have been found in the CYP genes from SNPs (the most abundant) to structural variations such as gene deletions, and duplications of genes and indels, creating gene products with variable activity depending on the impact of polymorphisms on enzyme function.[1]

To get a better idea of polymorphic CYP450 enzymes, Rodriguez-Antona and Ingelman Sundberg classify them into two classes (composed primarily of phase I metabolism enzymes):

- **Phase I-Class I.** Composed of the isoforms CYP1A1, CYP1A2, CYP2E1 and CYP3A4, which are well conserved across the species; they do not present significant functional polymorphisms and are active in the metabolism of xenobióticos.[22] However, polymorphic variants have been described that affect enzymatic functionality.[23]
- **Phase I-Class II.** Composed of more polymorphic isoforms, such as CYP2B6, CYP2C9, CYP2C19 and CYP2D6, which are active in drug metabolism.

  The enzyme that is best described as highly polymorphic is the isoform CYP2D6, from which > 100 variants have been described in the database http://www.cypalleles.ki.se/cyp2d6.htm.[24,25] More than fifty drugs are metabolized by this enzyme,[25] among which the most important is tamoxifen, which is used to treat breast cancer estrogen receptor carriers; this is the reason why the allelic variants have received much attention from the medical community.[22] The allelic variations found in the CYP2D6 gene, include SNPs and structural type variations, such as CNVs, from gene deletion to multiple copies (from 0 to more than a dozen).[26] This phenotypic alteration directly affects the phenotype and four different classes metabolizers have been found (Figure 2A):

  - Poor (gene deletion).
  - Intermediate (both alleles affected or deletion of one allele).
  - Normal (both functional alleles), and
  - Ultrafast (duplication and multiplication of functional alleles).[24]

Another gene that exhibits CNVs, is CYP2A6. Among the drugs that it metabolises is nicotine and its metabolite cotinine. Increased activity or the increase in the number of functional copies increases the risk of addiction to nicotine, and consequently the activation of carcinogenesis. For this reason, it is associated with cancer related to tobacco consumption.[26]

In addition to the variation found for phase I metabolism enzymes, the existence of genetic variation in phase II metabolism enzymes has also been documented:

- **Phase II.** This includes genes that encode phase II metabolism enzymes responsible for conjugation reactions. It has been documented that GSTM1, GSTT1, SULT1A1, SULT1A3 and UGT2B17 genes express CNVs, as the predominant form of genetic variation.[26] From this class of genes, thiopurine S-methyltransferase (TPMT) and dihydropyrimidine dehydrogenase (DPD), are the more representative models of therapeutic importance of the polymorphisms in this class of enzymes, since they are involved in the metabolism of antineoplasics.[6]

## GENETIC VARIATION REFLECTED ON TRANSPORTERS

Drug absorption occurs in the gastrointestinal tract and it was long considered a passive process that depended on the molecular weight, solubility, lipophilicity, concentration gradient and the pK of the drug, even the intercellular distance. It is currently known that absorption also occurs actively through the binding of molecules with transporter proteins, as well as against a gradient through membrane transport proteins or by protein channels that cross the membrane from the intracellular to the extracellular wall. These membrane and transmembrane proteins regulate cell homeostasis by importing and exporting endogenous or exogenous molecules.[27]

In drug therapy, several transporters are involved in the transfer of metabolites to their site of action. Among the most important are expression in the intestine (enterocyte), liver (hepatocyte), in membranes of the renal proximal tubule cells (kidney) and in cells of the blood-brain barrier, as well as intracellular expression, exemplified by those found in the central nervous system. Because of its location and function, these transporters are important throughout the LADME (liberation, absorption, distribution, metabolism and excretion) process, to which a drug is exposed when it enters the human body.[28]

The presence of polymorphisms in genes encoding these transport proteins may affect the synthesis or the three-dimensional conformation of the protein and result in a change in their affinity for the substrates. These changes have a major impact on therapy because they alter the drug concentrations at the site of action despite a standardized blood concentration.[27]

From the collection of genes in the human genome, it is thought that an estimated 500 to 1,200 encode carrier function proteins,[29] with MDR1 (multidrug resistance) transporters, OATPs (organic anion transporting polypeptides), OCTs (organic cation transporters) and OATs (organic anion transporters) as well as those that bind nucleosides being the most significant. These proteins move both endogenous and exogenous substrates among which are drugs.[27,28]

Of these transporters, P-glycoprotein (Pgp) was the first with which a description of the phenomenon of resistance to anticancer drugs was begun using experiments in cell lines from different tissues.[30] As a member of the family of "ATP-binding cassette" or ABC, Pgp requires energy (ATP) to carry out its transport function. The coding sequence of this protein is dictated by ABCB1 gene (alias MDR1) that is expressed primarily in intestine, kidney, liver and the blood-brain barrier, where it has a protective role,[30,31] given its ability to actively removes toxic agents, such as anticancer drugs from the cell cytosol to the extracellular fluid.[32] In addition to these, Pgp removes other compounds such as statins, immunosuppressants, and some viral protease inhibitors used in the treatment of various illnesses.[31] The absorption of these therapeutic agents may be restricted when Pgp expression increases since removal of these molecules to the extracellular fluid increases. As a consequence, bioavailability is reduced and this prevents reaching therapeutic plasma concentrations. In opposition to this event, decreased expression affects supratherapeutic concentrations of relevant drugs, which causes significant drug toxicity.[6]

The genetic variation shown by the MDR1 gene is mostly SNPs. For this gene, less than a hundred SNPs and a few indels along its sequence have been documented. The polymorphic variant that has been most studied is the C3435T in exon 26, which affects the expression and function of this transporter.

Another important transporter in drug therapy is the OATP-C, which is a product SCP1B1 gene. It is located primarily in the kidney and liver, which are the main routes of elimination of metabolic waste in the human body.[33] In a study with Repaglinide, it was reported that the genetic variant OATP-C*5 was associated with an increased area under the curve up to three times in comparison with individuals carrying the wild-type allele.[34]

## A GENOMIC VARIATION THAT IS REFLECTED IN OTHER PROTEINS THAT ARE TARGETED BY DRUGS

There are genes whose protein products are drug targets. These include receptors, ion channels and intracellular signaling proteins. They also exhibit polymorphisms in coding sequences, which can be decisive in therapeutic targets, as well as in the response to drugs.[6,27]

Since they are abundant on the cell surface, the receptors are crucial therapeutic targets. However, the genes whose sequence code for these GTP-binding proteins or protein G, have shown significant genetic variation, which goes beyond the success or failure of therapy. There is evidence of genetic variants in the CASR (calcium sensing receptor), in the angiotensin II receptor, in neurotransmitters

(dopamine, serotonergic and muscarinic receptor) and in the adrenergic (β-2 adrenergic) receptor to name the most important.[35]

The impact of these polymorphisms, range from the likelihood of conditions such as hypertension and hypercalcemia in the case of CASR, to predicting success of drug treatment for hypertension, as in the case of the angiotensin II receptor. The clearest examples of drug targets are the β1 and β2 adrenergic receptors, which are important in the treatment of asthma and heart failure.[35]

Another important example of a variant protein is vitamin K expoxide reductase best known in scientific circles as VKOR that has a major role in the response to anticoagulants, such as warfarin. This anticoagulant inhibits the carboxylation exerted by VKOR on the factors involved in the clotting cascade. This carboxylase activity enzyme is the result of the expression and translation of the VKORC1 gene. Changes along its coding sequence cause bizarre cases in terms of the response to warfarin. Such cases require doses of this drug, above the standard therapeutic range, to achieve an anticoagulant effect. Or simply cases where there is no response to any dose administered, with the consequence of thromboembolic or hemorrhagic events.[6,36]

## VARIABLE EFFECTS OF DRUGS

When an active substance enters a person's body, it can respond in different ways: either experiencing an effective response, a reduced response, simply no response to the therapy or some type of adverse reaction (ADR). Liver toxicity and cardiac arrhythmias are common adverse reactions that can lead to hospitalization and even death. Therefore, ADRs play a major role in morbidity and mortality of the world's population, resulting in the generation of significant costs for health systems.[27] According to analysis by Lazarou, *et al.,* in 1998,[37] it is estimated that ADRs are responsible for 6.7% of hospitalized patients and for 0.13% of deaths in hospitals in the United States. Pirmohamed, *et al.* found similar figures in a study that included hospitals in the United Kngdom.[38] For this reason, ADRs continue to be the biggest limitation for new drugs to reach the market. Also, they are the reason why a drug is removed from it. As a result of the above, researchers, healthcare providers, regulatory agencies such as the Food and Drug Administration (FDA), and the pharmaceutical industry, have directed their efforts to identify the factors that determine the risk for ADRs.[39] This multidisciplinary team has currently primarily focused their attention on genetic factors, since these are transcendental in the pathogenesis and prediction of ADRs. Being able to identify these factors will be crucial in drug therapy, since it will make the design of a treatment based on the individual's genetic profile possible, which will result not only in a better response, but also in a significant reduction in the number of ADRs (Figure 2B).[27]

## LABORATORY MODELS FOR PREDICTING DRUG EFFECTS

For years biotechnology has developed several *in vitro* models to study the metabolism of drugs, where liver microsomes assays, primary cultures of hepatocytes, cell lines and recombinant proteins, have been the best alternatives for dissecting the metabolic bases of drug actions and metabolism. Moreover, today we have a wealth of information about selective substrates and inhibitors, which make the precise and individual establishment of the functional capacity, for example of the different hepatic isoforms of CYP450 enzymes, possible. These, associated with the appropriate *in vitro* model for each particular case, conforms a wide range of useful tools in studies of drug metabolism (Figure 3).[40]

The pharmaceutical industry has seized on these tools, as well as microarrays (see below), to define individual genetic profiles and accelerate the identification and disposal of less suitable candidates for their pharmacologic trials.[41]

## SUBCELLULAR MODELS

Among the first models to evaluate the metabolism of xenobiotics, are liver microsomes. These consist of CYP450 proteins that are embedded in the membranes of the smooth endoplasmic reticulum of the liver. These are easily processed, since they are obtained by ultracentrifugation; their availability is not a big problem (if access for to liver tissue is not available, there are commercial alternatives) and they also have a high stability; in other words, they can be kept frozen for at least a couple of years. The HLM (human liver microsomes) contain Phase I metabolism enzymes, as well as some Phase II enzymes, which gives them good biotransformation ability. These properties have made this biological model the most popular.[40]

With the advancement in biotechnology, this system has evolved to another level, making the availability of microsomes generated from genetically engineered cells capable, of ensuring expression of a

single CYP450 enzyme. Therefore, the power to access different batches of well characterized microsomes creates the possibility of evaluating and analyzing the influence of certain factors, such as genetic polymorphisms.[42]

The primary limitation of this model is that it only contain enzymes anchored to membranes (mainly CYP450), excluding other types of proteins such as the cytosolic. As a result, only a partial reproduction of drug metabolism can be offered.[40]

## CELL MODELS OF NATURAL SOURCES

The disadvantages of using subcellular models with their partial metabolism can be corrected with the use of experimental models consisting of whole cells. Cells from different human tissues have been isolated and cultured, such as epithelial and liver cells, with the latter being the main metabolizers of xenobiotics. With the use of these primary cultures, the prediction of metabolism has improved, providing a scenario of what happens closer to the situation in vivo. However, the main drawbacks for the use of these cell models, is the provision of human tissue, coupled with the problem that the viability of primary cultures is short.[43]

## LABORATORY CELL MODELS

Through recombinant DNA technology, isolated cell lines can be genetically modified to be immortalized and remain viable for an indefinite period. This ensures their proliferation in culture, becoming an almost inexhaustible source of cells. The main obstacle of this model is their low Phase I metabolic capacity compared to primary culture. To solve this problem, the expression of a single enzyme, transferring the gene of the CYP450 enzyme of interest, can be favored in a stable and immortalized culture.[44]

Having a panel of cell lines for expression of a single enzyme makes this model a good candidate for evaluating the metabolism of new drugs.[43]

## BIOMARKER TESTING MODELS

During the different metabolic processes performed by the human body, metabolites that are intermediates or end products of metabolism are generated. Metabolomics is the measurement of endogenous endpoint metabolites in biofluids, such as serum, plasma, urine, or tears.

When a drug is given, it must be metabolized, so metabolic pathways are activated or deactivated, leaving as evidence, a change in the concentration of metabolites. These serve as markers to measure the efficacy and safety of drugs, as well as to select individuals to be treated.

For example, the FDA approved the labeling of Depacon® (valproate sodium injection). This drug slows the function of the neurotransmitter GABA, because of this feature it is used in the treatment of epilepsy and bipolar disorder. However, a side effect of valproic acid therapy is hyperamnios encephalopathy, a condition that has been observed in subjects who have disorders of the urea cycle. This alteration results in the accumulation of nitrogen (a waste product of protein metabolism), which, when accumulated, is transformed into ammonia, a highly toxic substance. Excessive ammonia accumulation can generate a uremic syndrome and even death. For this reason, the FDA recommends previous assessment of the urea cycle in patients undergoing treatment with Depacon.[45]

## "PROBE" DRUGS

The different isoforms of CYP450 enzymes responsible for the metabolism of endogenous molecules have a particular characteristic, a high substrate specificity and usually they catalyze only one type of chemical reaction. Contrary to this, the xenobiotic-metabolizing CYP450 (primarily families CYP1 to CYP3) are able to metabolize more than one substrate, even if their molecular structures are different; however, these enzymes at the same time present their own substrate selectivity.[45]

The human body is so amazing, that to ensure carrying out the neutralization and elimination of any xenobiotic that enters the body, a metabolite may be a substrate of different enzymes that can turn it into the same product or different metabolic products. This overlap in enzymatic functions allows that in case of the inhibition or inactivation of a particular CYP450 enzyme, other enzymes can replace it. In conclusion, xenobiotics tend to be biotransformed by several isoenzymes and few are actually metabolized by only one.

Much time was spent in searching for metabolic substrates that were unique to a particular member of the CYP450 family. This, in order to identify a set molecules whose oxidation or reduction could establish the enzyme responsible for its metabolism. It

should be clarified that with regard to drug-metabolizing enzymes, one cannot speak of absolute specificity, but one can of selectivity, which would be the more appropriate term. Based on this, information on substrates (drugs probes) is now available and their reactions are used together as tools to measure the activity of the major CYP450 responsible for drug metabolism in man (phenotyping).[46,47]

## DRUG PROBES AND BIOMARKERS

As technology advances, the development of biological models for studying drug-metabolizing enzymes and as a result the knowledge of the enzyme system has been growing steadily, reaching a blistering pace in the last 20 years. The wide versatility of these enzymes in the CYP450 family, that are able to metabolize many substrates and to catalyze a wide variety of transformations, make them unique proteins, that are not comparable with any other enzyme. That is why the study of their activity has focused not only on revealing the catalytic cycle or regulatory mechanisms, but also in knowing the keys to their variability.[48]

Probe drugs are metabolites (active components of drugs), which are well characterized with respect to which enzyme is responsible for their metabolism, which enzymes cause inhibition of their activity, which enzymes they induces, the type of chemical reaction that breaks them down and which products are derived from their metabolism. These are used in phenotyping (identifying metabolizer type), to evaluate the activity of an enzyme genotype of interest or in pharmacologic induction-inhibition assays.[49]

On the other hand, molecular markers can be used in the evaluation of clinical response, in the identification of risk of having a disease and as a guide for selecting the appropriate dosage, susceptibility to ADRs, resistance to treatment, and polymorphic drug targets.[45]

In most molecular biomarkers recognized by the FDA, probe drugs provide pharmacogenomic information without immediate recommendation for a specific action, with the use of this knowledge being an auxiliary of genetic diagnosis. With all the scientific evidence gathered to date, the FDA supports a list of molecular markers and probe drugs that are linked to pharmacogenomic data. These data are freely available and are posted on the FDA website (www.fda.gov/Drugs/ScienceResearch/ResearchAreas/Pharmacogenetics/ucm083378. htm.).

In addition to the information provided by the FDA, a database that includes all the constantly updated information about the CYP450 enzymes has recently been released. This database, called the SuperCYP database (http://bioinformatics.charite.de/supercyp), is a valuable source because it contains more than 1,170 drugs with more than 3,800 interactions, including references. It also describes approximately 2,000 SNPs and mutations that have been listed and ranked according to their effect on expression and/or activity, including the possibility of obtaining 3D images of the structures of the filed isoenzymes.[50]

## EXPERIMENTS WITH MODERN BIOTECHNOLOGY

In the past, screening of new molecules with potential pharmacological activity, was carried out with the *in vitro* study models mentioned above. This allowed assessment of the inhibitory potential of these molecules and the establishment of the enzymology of metabolism.[51]

At present, the major drug-metabolizing enzymes are well characterized, and even recombinant, produced in bacteria such as *Escherichia coli*, *Saccharomyces cerevisiae* yeast and in baculovirus from insect cells, among other biotechnological hosts. This biotechnology development has made the generation of new *in vitro* tests for predicting human metabolic clearance possible.[52]

Among the most important advantages of this laboratory model is that genetic variation can be incorporated into the expression of CYP450 (genotype-specific recombinant enzymes). It also allows assessment of the inhibitory potential of test metabolites and simultaneously the type of metabolic reaction for each of the enzymes in question. As an added value, these studies allow the calculation of the values of Vmax, Km and Ki, which are kinetic parameters of the enzymes in question, which are necessary for extrapolation *in vitro-in vivo*.[51]

With a battery of several recombinant enzymes, the enzyme responsible for the metabolism of recently developed drugs can be identified.[52] Recent studies have been conducted that compare the use of recombinant enzymes versus the use of human liver microsomes and cryopreserved hepatocytes. The results of these studies revealed that recombinant CYP450 enzymes provide an improved and reliable prediction, and offer a more sensitive test for drug clearance testing.[53]

## NEW PHARMACOGENOMIC TESTING

In this genomic era, several companies manufacture tests that determine the genetic profile of patients using DNA chips. The first test of this type that was ready for clinical use, was the "AmpliChip CYP450 test" by Roche, which was released in the United States in 2005. Using DNA chip or microarray technology (usually a microscope slide carrying tens or hundreds of thousands oligonucleotide probes that react with the genome of an individual to track mutations and polymorphisms in each patient), the test provides a report indicating the genetic variations present in CYP2D6 and CYP2C19, thereby predicting whether a patient is a poor, an intermediate, a normal, or an ultrafast metabolizer of substrates for each enzyme. This helps determine the metabolism involved in approximately 25% of prescribed drugs.[54] Another chip that is also already on the market is the DMET (drug metabolism enzymes and transporters) chip panel by Affymetrix designed for use in research and that evaluates polymorphisms in CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4, 5, 7 and transporters.[55,56]

On the other hand, a versatile DNAchip designed for both diagnosis and research is the PHARMAChip by Progenika Biopharma S.A., which allows the identification of 85 of the most relevant pharmacogenetic polymorphisms and best characterized in the international scientific literature, with a sensitivity and specificity greater than 99.9%. The PHARMAChip detects polymorphisms in cytochrome P450 genes and 25 genes involved in drug metabolism and transport. This test also provides a report that predicts the metabolizer phenotype in the patient for each of the genotypes revealed, thus allowing recommendations on the type and dose of drugs with which the disease can be best treated for a given patient, going from the pharmacogenetics to the pharmacogenomics era with personalized genomic medicine bioanalysis.[57] So far, this technology has been used in several studies to simultaneously evaluate several genes and markers. One of them revealed the ability of AmpliChip to parallelly identify several CYP2D6 genotypes and stratify them into four phenotypes, poor, intermediate or decreased, normal or extensive, and ultra-fast metabolizer. This test has been approved by the FDA.[58]

In 2009, using the Affymetrix DMET microarray platform, the impact of numerous genetic variants in the CYP450 genes with respect to the metabolism (the active metabolite concentration in blood) and efficacy (inhibition of platelet aggregation) of Clopidogrel was evaluated.[59] Also in 2010, in a study of genotype-phenotype correlation, multiple polymorphisms in the CYP450 genes and the transporter Pgp were evaluated in order to determine the genetic influence on the drug interaction of Tipranavir and Ritonavir.[55]

In Mexico there is already experience in using these new technologies, as one of the authors (HABS) leads a public-private consortium with funding from the Scientific and Technological Cooperation Agreement between Mexico and the European Union (FONCICYT), to test in Mexico PHARMAchip to assess its usefulness in predicting the efficacy and toxicity of anti-breast cancer drugs.[60]

## DRUGS WITH ACCOMPANYING TEST LABEL

The FDA has included a recommendation for genetic testing before prescribing certain drugs. The best example is that of warfarin, which is used in the treatment and prevention of thromboembolic events (of interest CYP2C9 and VKORC1).[58,61,62] Other drugs that include this label is Clopidogrel, a drug used to prevent atherothrombotic and cardiac events (interest CYP2C9),[59] Abacavir, which is an antiretroviral used in HIV therapy (gene of interest is HLA-B*5701);[63] tamoxifen, commonly used in breast cancer therapy (genes of importance are CYP2D6, CYP3A5 and SULT1A1); and 6-mercaptopurine, commonly used in the treatment of acute lymphoblastic leukemia (gene of interest is TPMT) among others.[58,61]

## CONCLUSIONS

Modern biotechnology and genomics have begun to unite to replace biochemistry and genetics with the desire in medicine to anticipate the response of patients to different treatments with pharmacochemicals and biotechnologicals. Although pharmacogenomic testing begins to influence the way in which medicine is practiced, the FDA recommends it only for certain clinical scenarios, especially cancer therapy. With the use of biotechnological tools, such as liver microsomes assays, cell models, molecular biomarkers in drug probe monitoring, recombinant protein and DNAchip testing, diverse factors at various levels that influence drug response can be evaluated. With all the genetic and metabolic information supplied by these predictive tools, the most effective therapy to combat disease can be provided.

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