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Pharmacogenetics: Matching the Right Foundation at Personalized Medicine in the Right Genomic Era

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1. Introduction

The aim of this chapter is to overview the promises of the pioneering field of pharmacogenetics towards personalized medicine, completely changing the present therapeutic paradigm of “one dose fits all patients” and “trial-and-error” prescriptions to “matching the right dose to the right, specific genetic signature of the patient and at the right time”. The review points out the evolution from pharmacogenetics to pharmacogenomics, as well as the impact of genome-wide-associated studies (GWAS) and next generation sequencing technologies on deciphering “missing heritability” and on validation and approval of pharmacogenetic biomarkers as it is reflected both in regulatory authorities recommendations and from consortia perspectives. Pharmacogenetics’ translation from bench towards clinical practice in personalized medicine and drug discovery and development underlies the increasing benefits of pipeline pharmacogenetics, especially in the high-priority domains, as well as the emergence of the electronic health records-, biobanking- and bioinformatics-driven pharmacogenetics within extended international networks. More effective and successful integration of pharmacogenetics in clinical practice should address challenges regarding bioethics, insurance and privacy, consensus scientific guidelines, education, pharmaeconomics and regulatory policy issues. Finally, there are illustrated the promising perspectives opened by: the interrogation of extensive electronic databases comprising clinical phenotype and genotype information; discovery of novel biomarkers by mining epigenoms, “junk DNA”, mitochondrial and RNA polymorphisms; and integration of nanotechnologies, in order to achieve the major objective of selecting the right therapeutic strategy endowed with the highest level of efficacy and safety among a predictable segment of the genotyped patients’ population.

2. Pharmacogenetics: Conceptual evolution to pharmacogenomics

2.1 Pharmacogenetics: Brief history and definition

The first clinical observations of interpatient variability in clinical response to standard therapeutic doses and the pioneering contributions of Sir Archibald Garrod, Arno Motulsky,
Friedrich Vogel and Werner Kalow led to the concept of pharmacogenetics. In “Inborn Factors of Disease” (1909), Sir Archibald Garrod noticed the implication of the “biochemical individuality” in the interpatient variability of the metabolism and efficacy of the standard regimens. In 1950s, there were described sensitivity to primaquin and the risk of hemolytic anemia of the patients with glucose-6-phosphate deficiency (G6PD), slow metabolizers/acetylators of standard doses of isoniazid among tuberculosis patients with increased risk of peripheral neuropathy, prolonged apnea after succinylcholine administration, in relation to hereditary factors. The involvement of genetic factors in the adverse drug reactions was mentioned for the first time by Arno Motulsky – considered the father of the pharmacogenetics – in his paper “Drug Reactions, Enzymes and Biochemical Genetics” (1957). The concept of pharmacogenetics was introduced by Friedrich Vogel in “Moderne problem der humangenetik” (1959) as the study of genetic determinism of the interindividual variability to drugs action. The first monography on pharmacogenetics „Pharmacogenetics: Heredity and the Response to Drugs” (1962) belongs to Werner Kalow, based on his pioneering work on the relation between genetic polymorphisms of butyrylcholinesterase and the risk of prolonged apnea to the standard therapeutic doses of succinylcholine. (Liewei et al., 2011; Grossman & Goldstein, 2009; Tepper & Roubenoff, 2009)

Pharmacogenetics correlates genetic factors to the interindividual variability in drug-response phenotypes and has mainly focused on the association between monogenic polymorphisms and the variation of the drugs’ metabolism. (Liewei et al., 2011)

Pharmacogenetics has the potential to increase the clinical benefit and reduce the risk of adverse drug reactions (ADR) in outliers, i.e. people whose drug responses are not “average”. (Woodcock & Lesko, 2009)

Pharmacogenetic studies involve the identification of genetic classifiers or markers used to predict interpatient variability concerning drugs’ efficacy and/or safety. These genetic markers could be generated through one of the following approaches: candidate gene approach, pathway-based approach or whole genome scan (also cited as Genome Wide Association Studies GWAS) approach.

2.2 Candidate gene, pathway-based and genome-wide studies

In the candidate gene approach, a panel of genes (candidate gene list) is generated based on the hypothesis in question to include drug target and mechanism pathway genes, as well as genes encoding the drug-metabolizing enzymes and membrane transporters involved in absorption, distribution, metabolism and elimination (i.e., drug pharmacokinetics). (Spraggs et al., 2009) The candidate gene approach has been applied by the majority of pharmacogenetics studies to detect associations between known single nucleotide polymorphisms (SNPs) and clinical or pharmacological end points, especially in the cases where there is a major drug metabolism or target gene that has a polymorphism that significantly changes its function. It is a hypothesis-driven approach that enables a study-design adjustment so as to acquire sufficient statistical power. The major drawback resides in its inconsistency in validating genetic markers, especially in cases where allelic variants are not highly penetrant, making the results of these studies difficult to interpret. (Wu et al., 2008 as cited in Sissung et al., 2010; Sissung et al., 2010)

The pathway-based approach uses foreknowledge of both genetic variants and the pathways in which they are involved, therefore this approach has proven particularly useful
in identifying and characterizing pharmacogenetics endpoints within studies aimed to test the interaction between genes. The major obstacles reside in: the necessity of machine learning techniques; complexity of studies requiring larger sample sizes than candidate gene approaches; difficulty to validate gene–gene interactions due to incomplete understanding of the fundamental biology of the pathways’ interactions. (Sissung et al., 2010)

The genome-wide approach is useful in determining the most significant SNPs associated with a phenotype amongst a high-density set of polymorphisms. GWAS are most useful to discover SNP associations where prior knowledge (i.e., mechanism, inheritance pattern, protein interactions and so on) is not available. Genome-wide association studies require a careful design of key issues like: a) type of study (case/control or continuous phenotype) according to the key-question and with a well-defined phenotype across all samples; b) sample sizes, dependent on specific effect size and type of study; c) population stratification between cases and controls; d) genotyping technology; e) raw data quality control and processing. (Wu et al., 2008 as cited in Sissung et al., 2010; Shianna, 2009)

GWAS are discovery-driven rather than hypothesis-driven, they evaluate multiple hypotheses and require large sample size, cost and computing power, often resulting in weak statistical signals and false positives (i.e., Type I error). Frequently, GWAS require a two-stage design where discoveries are made using a high-density SNP array and are then validated using additional patient sets and a more hypothesis-driven approach. (Wu et al., 2008 as cited in Sissung et al., 2010) Although recently GWAS application has dramatically increased, few studies have been published partially due to its primarily exploratory nature that requires further replication in large size and independent samples of the initial findings above the genome-wide significance and after correcting for multiple testing. For instance, in the case of antipsychotics drugs (ziprasidone, olanzapine, risperidone, iloperidone) GWAS revealed SNPs located in intergenic regions, but the functions of the variants on the drugs’ response are still unknown. (Jian-Ping Zhang, 2011)

The application of GWAS in a population-based cohort allows the study of all possible genetic determinants of a drug response’s phenotype in a hypothesis-free (i.e., unbiased) approach and is performed on commercially available, efficient and cost-effective high-throughput, genome-wide genotyping platforms (such as: Illumina’s Infinium BeadChips, Affymetrix GeneChips) targeting 100,000 SNPs, or 500,000 SNPs or even 1,000,000 SNPs of the genome. (Spraggs et al., 2009)

Functional SNPs for each selected gene are added based on a literature survey and especially by using a minimum set of “tagging SNPs” (tSNPs) sufficient to capture the common genetic variations (whose minor allele frequency is higher than 2-5%) which allow almost complete genome coverage for the most of genetic diversity in human populations. (Grossman & Goldstein, 2009) Tagging SNPs greatly increase the genomic coverage of genetic variability and they reflect the well-established patterns of linkage disequilibrium, making possible to genotype only the tagging SNPs in order to capture the content of other associated SNPs in the region. The HapMap database with its incorporated software was created by The International HapMap Project (2005) and is an appropriate publicly available resource for selecting globally useful “tagging SNPs” that has been implemented by both commercial companies and academic laboratories. Tagging SNPs are chosen mainly based on \( r^2 \) threshold, besides a variety of other criteria (ethnicity, SNP’s functional effects etc.). The \( r^2 \) threshold is the correlation coefficient between any observed marker and a putative
causal allele and is a study-independent measure of SNP utility, being considered a leading standard for evaluating performance of marker sets; the minimum customary pair-wise value for $r^2$ is 0.7-0.8. (Bakker, 2005 and Pe’er, 2006 as cited in Grossman & Goldstein, 2009)

There are currently commercially available arrays (SNP chips) that contain close to 1 million tagging SNPs with excellent genomic coverage for most populations as defined in HapMap Project. For example, Illumina HumanMap 300 has essentially the same statistical genomic coverage as the Affymetrix Mapping 500 K Set, while the Illumina HumanMap 550 array is statistically superior for GWAS. (Hirschhorn & Daly, 2005; Barrett & Cardon, 2006; Pe’er et al., 2006, as cited in Shianna, 2009)

2.3 Pharmacogenomics’ scope and goals

The elucidation of the sequence of the human genome in 2001 and the identification and analysis of functional elements in the human genome by the ENCODE (ENCyclopedia Of DNA Elements) Project represented major steps towards a more comprehensive characterization of all functional elements in the human genome. Moreover, the HapMap Project aims to generate a haplotype map of the human genome, describing the common patterns of the human genetic variation which would affect complex, multigenic diseases and responses to drugs and environmental factors. The emergence of the term pharmacogenomics was possible after the availability of the human haplotype map (HapMap) and of high-throughput genotyping platforms that have been facilitating more systematic genetic screens for new and clinically important drug targets. (Passetti et al., 2009) Therefore the concept of pharmacogenomics has progressively evolved from pharmacogenetics and expands beyond monogenic pharmacokinetics traits, making also the transition from associative genetic studies based on candidate gene hypothesis towards genome-wide association/screening studies (GWAS) in order to identify genetic biomarkers with prognostic role for disease progression and predictive capacity for drug responsiveness.

The evolution from pharmacogenetics to pharmacogenomics was due to: a) the integration of –omics technologies and bioinformatics into the genomic medicine and systems pharmacology; b) the acquisition of catalogued genomic and clinical data bases (such as “Pharmacogenetics and Pharmacogenomics Knowledge Base”, „Connectivity Map”, International HapMap Project); c) identification of SNPs that ‘tag’ much of the common haplotype variation across any genomic region of a given population; d) positive genetic associations studies between specific genetic signature of patients and variations to standard therapeutic regimens; e) analytical and clinical validation of genetic biomarkers predictive for drug response. (Ayslin et al., 2009)

According to the US Food and Drug Administration (FDA)-approved definitions, pharmacogenetics is ‘the study of variations in DNA sequence as related to drug response’ (Liewei et al., 2011), and currently is regarded as a subdomain of the much more comprehensive pharmacogenomics that is FDA-defined as ‘the study of variations of DNA and RNA characteristics as related to drug response’. (Trent, 2010)

Pharmacogenomics studies the differential expression profiles at the level of the entire human genome in complex interaction to drugs, in a systemic and integrative manner. The identification of all the genetic and epigenetic differences that are the cause of phenotypic variations in patients’ responsivity to therapy is a major objective in pharmacogenomics. (Passetti et al., 2009) Each person’s phenotype is best determined by the paired combination of
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the genome and the epigenome. Epigenetics and epigenomics refer to the study of factors that affect gene (or, more globally, genome) function, but without an accompanying change in genes. Typical epigenetic factors might be illustrated by changes in DNA methylation or in chromatin that modify genome structure and hence influence gene expression even in the absence of variations of DNA sequence. (Willard, 2009) Therefore, to achieve the main goal of therapy individualization, pharmacogenomics should also evaluate genetic variation in the context of the individual: gene–gene, gene–drug and gene–environment interactions which might influence the course of a disease and the response to treatment. (Passetti et al., 2009)

Pharmacogenomics is the interface between genomic medicine and systems pharmacology, the two essential pillars supporting the gateway to personalized medicine. (Aislyn et al., 2009) The transdisciplinary field of genomic medicine refers to the use of large-scale genomic information and to the consideration of the full extent of an individual’s genome, proteome, transcriptome, metabolome and/or epigenome in the practice of medicine and medical decision-making. Genomic medicine’s approaches include gene expression profiling to characterize diseases and define diseases’ prognosis, genotyping variants in genes involved in drug metabolism or action in order to select the correct therapeutic dosage for an individual, scanning the entire genome for millions of variants that influence an individual’s susceptibility to disease, or analyzing multiple biomarkers to monitor therapy and to provide predictive information in presymptomatic individuals. (Willard, 2009) Genomic medicine brings together knowledge on the relationships between genetics, pathophysiology and pharmacology, thus forming the base for systems pharmacology. Experimental and computational approaches enable systems pharmacology to provide holistic, mechanistic information on disease networks and drug responses, and to identify new drug targets and specific drug combinations. Network analyses of interactions involved in pathophysiology and drug response across multiple scales of organization, from molecular to whole organism, will allow the integration of the systems-level understanding of drug action with genomic medicine, thus generating the personalized medicine. (Aislyn et al., 2009) Personalized medicine refers to a rapidly advancing field of health care that takes into account each person’s unique clinical, genomic and environmental information. The goals of personalized medicine are to optimize preventive health care strategies and outcomes of drug therapies for each individual, while people are still healthy or at the earliest stages of disease, by an unprecedented customization or tailoring of medication types and dosages and/or prophylactic measures. (Willard, 2009)

The great promise of pharmacogenomics towards personalized medicine resides mainly in generating an individualized therapeutic guide, highly predictive for much safer and more efficient drug and doses choice for an accurately predicted, homogenously genotype-segment of patients who are responders to the treatment, rather being focused around each individual specifically. (Grossman & Goldstein, 2009)

Pharmacogenomics aims to individualize therapy on the patient’s specific genetic profile, by matching the right drug to the right patient at the right time. Pharmacogenomics’ translation from bench into clinical practice is broadening the perspective of personalized medicine so as in the near future we might rely on a “DNA chip”/”pharmacogenomic card” specific to each patient and on each genotype preemptively recorded in electronic medical records in order to individualize both the diagnostic procedures and the safest and most efficient medications prior to treatment initiation.
3. Pharmacokinetic pharmacogenetics

Relevant allelic variants to drug treatment’s outcome have been discovered in the genes encoding enzymes and transporters involved in drug pharmacokinetics: absorption, distribution, metabolism and excretion (ADME). Enzymes involved in the biotransformation of xenobiotics are classified as phase I or phase II. Phase I enzymes catalyze hydrolysis, reduction and oxidation reactions, while phase II enzymes catalyze conjugation reactions such as sulfation, acetylation and glucuronidation. (Sissung et al. 2010). The majority of phase I reactions are catalyzed by the cytochrome P450 (CYP) enzymes. There are 57 cytochrome P450 (CYP) genes and about the same number of pseudogenes, which are grouped according to their sequence similarity into 18 families and 44 subfamilies. However, only three of those families, CYP1, CYP2 and CYP3, catalyze most phase I reactions of drugs; over 75% of prescribed drugs are metabolized at least in part by three subfamilies: CYP3A, CYP2D6 and CYP2C. (Zanger et al., 2008; van Schaik, 2008, as cited in Sissung et al., 2010) Phase II reactions significantly enable the excretion of drugs by considerably increasing the hydrophilicity of the substrate or deactivate highly reactive species. Key phase II enzymes include N-acetyltransferases 1 and 2 (NAT1 and NAT2), thiopurine S-methyltransferase (TPMT), and the uridine diphosphate glucuronosyltransferase (UGT) family; polymorphisms in these genes have been shown to have clinical implications for a variety of diseases. (Zhou et al., 2008, as cited in Sissung et al., 2010)

3.1 Pharmacogenetics of drug-metabolizing enzymes

Biotransformation of the 200 most often prescribed drugs is catalyzed by members of the CYP3A family (37% of the drugs), followed by CYP2C9 (17%), CYP2D6 (15%), CYP2C19 (10%), CYP1A2 (9%), and CYP2C8 (6%), while CYP2B6 and other CYP isoforms (CYP2A6 and CYP2E1) participate in the metabolism of 4% and 2% of the drugs, respectively. The clinically well-established polymorphisms of CYP2C9, CYP2C19, and CYP2D6 genes are involved in approximately half of these top 200 drugs, since many of the drugs used in high-prevalence diseases in the Western countries are known to be metabolized by these CYPs. (Zanger et al., 2008)

CYP2C9, highly expressed in liver, metabolizes many weakly acidic substances like the anticoagulant warfarin, the anticonvulsants phenytoin and valproic acid, cardiovascular drugs like rosuvastatin and losartan, and several nonsteroidal anti-inflammatory drugs (NSAIDs). Many of these drugs have a narrow therapeutic index, and variations in CYP2C9 activity are among the recognized factors for adverse drug reactions. In vitro and clinical studies have consistently demonstrated that the CYP2C9*2 and *3 alleles are associated with significant, but highly variable, reductions in intrinsic clearance depending on the particular substrate; for instance, CYP2C9*3 allele might be associated to up to 90% reduction in the enzymatic activity of the CYP2C9 protein. The prevalence of CYP2C9*2 and *3 alleles is 35% in Caucasians and much lower in black and Asian populations. Carriers of CYP2C9*2 and CYP2C9*3 alleles are poor metabolizers and have high plasma levels due to low clearance of the substrate-drugs, therefore they experience higher incidences of adverse drug reactions like hypoglycemia from antidiabetic drugs, gastrointestinal bleeding from NSAIDs, and serious bleeding from warfarin treatment. (Pilotto et al., 2007, and Flockhart et al., 2008, as cited in Zanger et al., 2008)
The CYP2C19 isozyme metabolizes preferentially proton-pump inhibitors (PPI) like omeprazole and pantoprazole indicated in gastroesophageal reflux disease, gastric and duodenal ulcer. The poor metabolizer (PM) phenotype results from two null alleles, leading to the absence of functional CYP2C19 protein, whereas extensive metabolizers carry at least one functional allele. The prevalence of null alleles is about 3–5% to white and black populations, whereas up to 20% of Asians are carriers of two null alleles. The two most common null alleles are CYP2C19*2 occurring exclusively in Caucasians, and CYP2C19*3 occurring primarily in Asians. The PPI-efficacy depends on the plasma concentrations achieved over time, which are strongly influenced by CYP2C19 gene polymorphisms. PM subjects who are carriers of null alleles benefit from their lower metabolism rate because their drug levels are maintained higher for longer periods. On the contrary, subjects with the CYP2C9 *1/*1 wild-type genotype should receive higher doses of these PPIs in order to achieve stronger acid suppression compared to *1/*2 and *2/*2 subjects. (Kawamura et al., 2007, as cited in Zanger et al., 2008; Zanger et al., 2008; Tomalik-Scharte et al., 2008)

The clinical consequences of pharmacokinetic variability associated to genetic polymorphisms of CYP2D6 gene for tricyclic and selective serotonin re-uptake inhibitors antidepressants, beta-blockers, anticancer agent tamoxifen, as well as to the CYP2C9 genetic variants for AT1 (angiotensin II type 1) receptor antagonists (sartans), and anticoagulants (warfarin, acenocoumarol, phenprocoumon), are covered in other chapters of the book.

The CYP3A4 subfamily contributes to the metabolism of the most diverse group of substrates of all human P450s, as their active sites are flexible enough to bind and metabolize many preferentially lipophilic, structurally large compounds, such as: the immunosuppressants cyclosporin A and tacrolimus, macrolide antibiotics like erythromycin, anticancer drugs like taxol, benzodiazepines, hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors like simvastatin and atorvastatin, and anesthetics. In addition, CYP3A4 is the only human drug-metabolizing P450 that shows a significant sex difference, in that women express approximately 1.5- to 2-fold more CYP3A4 and have higher in vivo clearance of several typical CYP3A4 drug substrates than men. Although a number of large-scale sequencing and phenotype–genotype correlation studies have been carried out, the functional effects of CYP3A4 gene polymorphisms on drugs pharmacokinetic variability remain controversial. (Zanger et al., 2008) However, CYP3A4 basal and inducible expression phenotype might be influenced by other genes, such as: a) multiple drug resistance gene MDR1 whose 2677T (Ser893) allele induced higher basal CYP3A4 expression and activity, whereas the 2677G allele showed a higher rifampicin induction ratio in primary hepatocytes; b) pregnan X receptor PXR gene polymorphisms mostly located in promoter or intron 1 regions associated with CYP3A4 basal and inducible expression levels. (Liu, 2007 and Lamba, 2008, as cited in Zanger et al., 2008)

N-acetyltransferase type 2 (NAT2) is a phase II drug metabolizing enzyme responsible for hepatic bioconversion of major antituberculosis agent isoniazid to acetylisoniazid. Isoniazid is a pivotal agent in the treatment of tuberculosis, that remains a global emergency due to the growing prevalence of drug-resistant Mycobacterium tuberculosis and of HIV infection. NAT2 gene is affected by a bimodal distribution polymorphism (acetylation polymorphism) described after clinical observation of more frequently and more severely peripheral neuropathy and hepatotoxicity as adverse drug reactions to the slow-acetylators patients. These patients have mean elimination half-lives of 180 min. in comparison with 80 min. for rapid-acetylators. Carriers of at least one wild-type allele (NAT2*4) or a high-activity variant
allele (NAT2*12) have proven high NAT2 enzymatic activity (rapid acetylators), whereas those with two low-activity variants are slow acetylators. Rapid acetylators are more prevalent in East Asia (58-90%) than in Europe (32-43%). Tailoring isoniazid therapy means to increase isoniazid dose to rapid acetylators so as to achieve therapeutic efficacy and to reduce the dose administered to slow-acetylators so as to avoid adverse drug reactions while maintaining the desired antituberculosis effect. (Tomalik-Scharte et al., 2008)

Pharmacogenetics of other phase II drug-metabolizing enzymes is discussed in detail in other chapters for: thiopurine-S-methyl-transferase (TPMT) polymorphisms and the necessity to individualize the therapeutic doses of mercaptopurine and azathioprine; dihydropyrimidine dehydrogenase deficiency syndrome noticed during the treatment with standard doses of fluoropyrimidines (5-fluorouracil) to a segment of cancer patients carriers of certain genetic mutations; the pharmacokinetic variability and increased risk of myelotoxicity noticed for the anticancer drug irinotecan as a consequence of genetic polymorphism of UDP-glucuronosyltransferase (UGT1A1).

3.2 Pharmacogenetics of transporters

Transporters play a critical role in ADME because they are involved in the efflux and/or influx of drugs via active transport or facilitated diffusion, thus transporters affect drug uptake, bioavailability, targeting, efficacy, toxicity and clearance and they should be considered in combination with metabolic enzymes when discussing drugs’ outcomes. Two types of transport superfamilies, ATP-binding cassette (ABC) proteins generally acting as efflux pumps and solute-linked carrier (SLC) proteins as typically influx transporters, are responsible for the majority of drug and endogenous substrates transport. Many transporters have a broad range of substrates; for instance, ABCB1, also known as P-glycoprotein and MRD1, transports several classes of drugs, including anticancer agents, antibiotics, immunosuppressants and statins. (Sissung et al., 2010)

Largely as a result of the Human Genome Project, great advances in molecular biology, sequencing methods and availability of genome-wide technologies, genetic variants across the entire genome, including coding and noncoding regions of multiple transporter genes were identified, functionally characterized and associated with various drug-response phenotypes. Thus, functionally relevant polymorphisms were discovered for the members of ABC and SLC superfamilies of transporters and have been widely studied with positive associations to individual susceptibility to drug-induced adverse events, to variations in drug plasma levels, or renal clearance. (Yee SW et al., 2010) For instance, the organic anion transporting polypeptide polymorphism SLCO1B1*5 (Val174Ala, c.521T>C) is associated with variability in response to statins (atorvastatin, pravastatin, pitavastatin, rosuvastatin, simvastatin), repaglinide, fexofenadine and methotrexate. The SLCO1B1 genotype affects the transport function and may predict, in a substrate-dependent manner, the attenuated lipid-lowering response to statin therapy. Moreover, stronger evidence has been provided for the role of the SLCO1B1 genotype in predicting the development of myopathy among patients receiving simvastatin in 40 mg doses. (Yee SW et al., 2010; Romaine et al., 2010) Prescribing relatively low dose of simvastatin to those who are heterozygous for the high risk allele SLCO1B1 could reduce the incidence of myopathy by nearly 60%, while avoiding simvastatin only to those who are homozygous for the risk allele (nearly 2% of the population analyzed by the SEARCH group) could reduce the incidence of myopathy by 25%. Further investigation is required to identify the optimal therapeutic approach. (Nakamura, 2008)
Furthermore, organic cationic transporter OCT1 (R61C, P160L, G401S, 420del and G465R) and OCT2 (A270S) polymorphisms are associated with response’s variability to metformin, cisplatin and imatinib. The ABCG2 genotype (rs2231142, Gln141Lys, c.421C>A) is associated with variable pharmacokinetics parameters of atorvastatin, rosuvastatin, gefitinib, sulfasalazine and diltomotecan. ABCB1 gene polymorphisms (c.3435C>T, 2677G/T/A) and/or ABCC2 (-24C>T, c.1249G>T, c.3972C>T, c.4544C>T) are related to therapeutic and adverse effects to anticancer, antiviral and/or antiepileptic drugs. (Yee SW et al., 2010) SNPs of ABCB1 were found to be associated with moderate-to-severe neutropenia, hand-foot syndrome and diarrhoea in colorectal patients treated with capecitabine or 5-fluorouracil. (Gonzales-Haba et al., 2010)

Nonsynonymous (coding) SNPs generally appear to affect the expression level of the transporter on the plasma membrane and the transporter function in a substrate-dependent manner. In comparison to nonsynonymous variants, noncoding region variants (such as in the proximal promoter region) are more abundant, minor allele frequencies being often higher and the functional consequences are more modest and highly dependent upon the haplotype. The frequency of noncoding polymorphisms are greater in ABC transporters highly expressed in the liver than in SLC predominantly expressed in the kidney. The projected functional map of the ‘transporter genome’ will characterize gene regions (enhancer regions upstream, downstream and intronic regions of transporter genes) having relevant functional variants and it will be superimposed on the genetic variants resulted from the 1000 Genomes Project in multiple ethnic populations. (Yee SW et al., 2010)

3.3 AmpliChip™ CYP450 test

In 2005, the FDA approved the first pharmacogenetic test AmpliChip™ CYP450 Test (Roche Molecular Systems, Inc., NJ, USA) based on Affymetrix (CA, USA) microarray technology for genotyping 27 alleles in CYP2D6 and three alleles in CYP2C19 genes associated with different metabolizing phenotypes. The test is recommended for the assessment of the patient’s metabolizing status for each drug that is a substrate for CYP450 isoenzymes 2D6 and 2C19 and for the dose adjustments in outlier patients who are either ultrarapid- (UM) or poor-metabolizers (PM), in order to achieve the therapeutic efficacy and to avoid the risk of severe adverse reactions. (Squassina et al., 2010) Key genetic mutations associated with clinical relevance on drug plasma concentrations and risk of either lack of efficacy for UM or adverse reaction for PM, are used to predict the metabolizer phenotype (ultrarapid, extensive, intermediate and poor metabolizers). FDA has included these biomarkers only as informational pharmacogenetic tests on labels of drugs mainly metabolized by these pathways, such as: CYP2C19 genotyping for prasugrel, voriconazol, (es)omeprazole, and genotyping CYP2D6 for tamoxifen, atomoxetine, fluoxetine, paroxetine, amitriptyline, aripiprazole, risperidone, codeine, tramadol, timolol, propranolol, carvedilol. The purpose of these informational pharmacogenetic tests is to improve drug safety by dose optimization based on genotypes predictive for poor- or ultrarapid-metabolizer status, as well as to avoid high plasma concentration when co-administered with other drugs which are CYP2D6 strong inhibitors. (Squassina et al., 2010)

For instance, patients with poor metabolizer phenotype associated to null alleles CYP2D6*4, *3, *5, or CYP2D6*6, will have higher risk of the adverse reaction tardive diskinesia at standard doses of antipsychotics. The individualized doses for these PM patients will be reduced than standard regimens designed for wild-type normally functional allele. On the
contrary, to achieve the required level of therapeutic efficacy, ultrarapid metabolizers who are carriers of CYP2D6*Nxn multiple functional alleles will be treated with higher doses than those standard recommended for the “average” patients population able to normally metabolize the drugs. However, mothers with phenotype of ultrarapid metabolizers will rapidly convert standard doses of codeine into morphine thus increasing the risk of CNS depression of their breast-fed babies; in such cases of prodrug bioconversion, dose optimization requires to be lower than the standard dose. (Loo et al., 2010)

Although it is widely available in commercial labs, the AmpliChip™ CYP450 Test has still a limited clinical value because it is expensive (over $600/test), time-consuming (i.e. about two weeks), and yet there are no prospective study to demonstrate the cost-effective benefit of genotyping patients and selecting and dosing antipsychotic drugs accordingly. (Jian-Ping Zhang, 2011)

3.4 DMET Plus Panel genotyping platform

While the field of pharmacogenetics is moving towards exploratory, large-scale analyses of the interaction between genetic variation and drug treatment, the Drug Metabolizing Enzymes and Transporters DMET Plus Panel (Affymetrix) genotyping platform has proven a significant research tool. The DMET Plus Panel platform is a low- to mid-scale pathway-based, hypothesis-driven and exploratory pharmacogenetic approach, which interrogates 1936 genetic variations (copy-number variations, insertions/deletions, biallelic and triallelic single nucleotide polymorphisms SNPs) in 225 genes involved in the absorption, distribution, metabolism and elimination (ADME) of a very wide range of therapeutics, as well as a number of genes which regulate intracellular processes that facilitate ADME through indirect relationships, thus comprising biomarkers for all FDA-validated genes and included in the drugs’ label. (Sissung et al., 2010; Squassina et al., 2010)

The DMET Plus Panel platform is particularly useful for standardization of exploratory pharmacogenetics and for improvement of the clinical trials’ design conducted on smaller patient populations, having more variable end points and polygenetic traits, enabling increased statistical power and reduction of type I error (i.e., false positive) of GWAS. In addition, the application of DMET Plus Panel platform in phase I early clinical trials could identify the polymorphisms consistently associated with drugs’ pharmacokinetic variations, determine the recommended dose for later phase II and phase III trials based on genetic profile, thus reducing the attrition rate for new investigational agents. Moreover, besides detection of more common genetic variants, DMET is able to interrogate core biomarkers with an average minor allele frequency below 9%, in comparison with other SNP detection methods for minor alleles with an average frequency of 20%. However, the DMET Plus Panel platform’s utility resides mainly in the research setting, since it is not yet FDA approved, not customizable, does not include polymorphisms in many drug targets or in genes that are related to environmental exposures that could influence drug metabolism, and requires prospective clinical validation in order to translate the results in pertinent personalized medicine. (Sissung et al., 2010; Squassina et al., 2010)

Although genetic variation in ADME genes is essential in personalizing therapy, polymorphisms in genes not directly responsible for drug metabolism or transport, but regulating expression of many genes that encode transporters and phase I and phase II
enzymes, play critical roles in patients’ response to treatment. For example, many SNPs in
the nuclear receptors pregnane X receptor (PXR) and constitutive/active androstane
receptor genes alter the expression levels of \textit{ABCB1}, \textit{ABCC2}, \textit{CYP2C8}, \textit{CYP3A4}, \textit{UGTs} and
sulfotransferases (\textit{SULTs}) genes and contribute to variability in drug efficacy and safety.
(Sissung et al., 2010)

4. Pharmacodynamic pharmacogenetics

Pharmacodynamics can be defined as the study of the biochemical and physiological
effects of drugs and their mechanism of action. The effects of drugs result from their
interaction with macromolecular components of the organism – receptors, which are
grouped in a wide range of structural and functional families. The receptor occupancy by
a particular drug class triggers biochemical cascades in target cells and modulates diverse
intrinsic signaling pathways and functions, explaining the pharmacodynamic effect. (Ross
& Kenakin, 2006)

In addition to genetic polymorphisms of ADME genes, the clinical outcome of standard
therapeutic drug regimens is influenced by genetic variations in genes encoding drug
targets (receptors, enzymes, ion channels, neurotransmitter’s transporter) and pathways
affected by drugs.

Since pharmacogenetics of heart diseases therapy, anticoagulants, asthma medication,
anticancer agents including thiopurines, antidepressants, osteoporosis and antimalaria
drugs, represent other distinct chapters of the book, in order to avoid overlapping
information, this chapter will illustrate some functionally relevant polymorphisms of the
drug target genes and their role in the interindividual variability of drugs’
pharmacodynamics, in a complementary manner to the aforementioned issues.

4.1 Pharmacogenetics of drug hypersensitivity: Human Leukocytes Antigens (HLA)
system

In the field of highly active antiretroviral therapy (HAART), abacavir has created a
translational roadmap for a pharmacogenetic biomarker from discovery to a test used in real
clinical practice. The strong association between abacavir hypersensitivity reaction and
\textit{HLA-B*5701} genotype has been demonstrated in both observational and blinded
randomized clinical trials in racially diverse populations and represents the best example of
the clinical utility of pharmacogenetic screening in HIV medicine. (Mallal et al., 2008)

Hypersensitivity reaction to abacavir was observed during the clinical development
program in approximately 5–8% of patients. Hypersensitivity reaction (HSR) symptoms
appear early and resolve upon discontinuation of the drug, but worsen (and can be life-
threatening) with continued drug administration. Development of an abacavir skin patch
assay enabled refinement of the hypersensitivity reaction phenotype. The biomarker \textit{HLA-
B*5701}, validated in retrospective and prospective studies, was recommended in the drug
label in the EU and USA. The prospective screening for this biomarker in Caucasians as high
risk population allows a reduction in HSR frequency from 7.8% to 3.4%. (Trent, 2010)
The increased benefit–to–risk ratio and the economic consequences of \textit{HLA-B*5701}
pre-screening of HIV-infected patients before abacavir treatment’s initiation were
demonstrated in a prospective double-blinded clinical trial sponsored by GSK. (Mallal et al., 2008; Roses, 2009; Phillips et al., 2011)

Moreover, consistent data support the association of the HLA class II allele HLA-DRB*0101 with an increased risk of nevirapine-induced hepatotoxicity, as well as genotype-related peripheral neuropathy, hyperlipidaemia, lipodystrophy to HAART, nucleoside reverse transcriptase inhibitors-related pancreatitis and tenofovir-associated renal proximal tubulopathy. (Tozzi, 2010)

Other recent examples of important HLA associations with drug hypersensitivity include HLA-B*1502 and Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) that are associated with carbamazepine in Han Chinese; HLA-B*5801 and SJS/TEN and drug-induced hypersensitivity syndrome/drug reaction with eosinophilia and systemic symptoms associated with allopurinol; HLA-B*5701 and flucloxacin-induced liver injury. These pharmacogenetic associations hold the promise to convert the severe and adverse drug reactions into predictable and preventable ones in the future. (Phillips et al., 2011)

4.2 Pharmacodynamic pharmacogenetics of antipsychotics

Pharmacogenetic investigations of schizophrenia susceptibility loci and genes controlling drug target receptors, the blood–brain barrier systems, and epigenetic mechanisms could lead to a molecular classification of treatment response and adverse events of psychotropic drugs. It is estimated that more than 70% of patients with chronic schizophrenia discontinued their antipsychotic drugs, owing to poor effectiveness or tolerability. Most of the pharmacodynamic pharmacogenetic studies in schizophrenia have evaluated treatment response using the candidate gene approach.

The most relevant associations of genetics variants and antipsychotic clinical response were found for −141C Ins/Del in dopamine receptor gene DRD2, A-1438G in 5-hydroxytryptamine/serotonin receptor gene HTR2A, His452Tyr in HTR2A gene, Taq1A in DRD2, Ser9Gly in DRD3, T102C SNP in HTR2A, C759T SNP in HTR2C gene. For instance, patients who carry one or two Del alleles tend to have less favorable antipsychotic drug responses than patients with the Ins/Ins genotype in DRD2 −141C Ins/Del SNPs. Patients with G/G genotype for the HTR2A A-1438G polymorphism were less likely to respond to clozapine, olanzapine and aripiprazole, especially in negative symptoms, than other polymorphisms at this HTR2A locus. For the His452Tyr HTR2A genetic variants, the Tyr/Tyr genotype predicted poor response to clozapine. Higher risk of the adverse reaction tardive diskinesia was found for carriers of either: A2/A2 genotype at DRD2 Taq1A locus, Gly allele at DRD3 Ser9Gly, or C allele for 5HT2A T102C. (Jian-Ping Zhang, 2011)

Furthermore, single-nucleotide substitutions in the promoter region of serotonin receptor type 2C (5-HT2C) could be associated with antipsychotics-induced weight gain and metabolic abnormalities in Han Chinese patients treated over a 10-week period. The C/C genotype from 5HT2C SNP C759T was associated with higher weight gain to clozapine and olanzapine. Comparable results were found in Caucasians treated 9 months with these anti-
psychotics: carriers of the -795T variation gained less weight than study participants without this allele. (Broich & Moeller, 2008)

A repeat length polymorphism of the gene encoding the serotonin transporter, 5-HTTLPR, involves insertion/deletion of a 44-bp segment located upstream of the transcription start site in the promoter region; patients carrying the long allele are about twice as likely to respond to treatment at 4 weeks and reach remission, and less likely to suffer from side effects, than patients with the short/short genotype; short allele is associated with poor response to clozapine and risperidone treatment. (Jian-Ping Zhang, 2011) Moreover, insertion/deletion polymorphism in the promoter region of the serotonin transporter gene is also associated to clinical phenotype response to the antidepressants citalopram, paroxetine and fluoxetine. (Yee SW et al., 2010)

4.3 Pharmacodynamic pharmacogenetics of antidiabetics

Thiazolidinedione drugs (pioglitazone, rosiglitazone) promote the binding of the transcription factor peroxisome proliferator-activated receptor-\(\gamma\) (PPAR-\(\gamma\)) to its DNA response element. Thiazolidinediones promote adipocyte differentiation, increase insulin-stimulated glucose uptake into muscle, insulin suppression of hepatic glucose output, and insulin-stimulated lipolysis. The genetic variation Pro12Ala at the PPARG gene (encoding PPAR-\(\gamma\)) influences the clinical outcome: those carrying the Ala allele have a greater response to rosiglitazone, as well as a lower risk of edema after farglitazar or ragaglitazar therapy, than Pro/Pro homozygotes. (Kang ES et al., 2005 and Hansen et al., 2006, both cited in Pearson, 2009) The hypoglycemic effect of rosiglitazone might also be influenced by adiponectin gene ADIPOQ polymorphisms SNP +45T/G and SNP +276G/T: homozygotes G/G at +45 or +276 have a smaller clinical benefit. (Kang ES et al., 2005 as cited in Pearson, 2009) Sulfonylureas (tolbutamide, glimepiride, glibenclamide) bind to the SUR1 moiety of the pancreatic \(\beta\)-cell K\(_{ATP}\) channel causing the channel to close and triggering insulin secretion. The clinical efficacy of sulfonylureas seems to be associated to genetic variations in the KCNJ11 gene (encoding the Kir6.2 subunit of the K\(_{ATP}\) channel) and the ABCC8 gene. (Pearson, 2009) The polymorphisms ABCC8 Ser1369Ala and KCNJ11 rs5210 and E23K are in strong linkage disequilibrium and significantly associated with variations in fasting plasma glucose levels induced by sulfonylureas. (Feng Y et al., 2008, Glyon AL et al., 2003 and Sesti G et al., 2006, all cited in Pearson, 2009)

4.4 Pharmacogenetics of hepatitis C virus therapy

Response to pegylated interferon-alfa (PEG-IFN) and ribavirin (RBV) therapy in chronic infection with hepatitis C virus (HCV) is variable and a sustained virological response (SVR) is dependent on genetic factors, hepatitis C viral load, patient age, sex, weight, liver fibrosis stage, and adherence to therapy. Strong predictive, clinically relevant effect of IL28B genotype on SVR shows that C/C genotype at rs12979860 has a greater HCV-genotype 1 RNA decline from days 0-28 than patients with the C/T or T/T genotype. The IL28B genotype may also be considered in conjunction with virological response after 4 weeks: thus, patients with poor viral kinetics and T/T genotype at rs12979860 may decide to stop therapy. In North America, a commercial test for IL28B genotyping is now available and costs approximately $300. (Afdhal et al., 2011)
5. Pharmacogenetics’ translation from bench towards clinical practice in personalized medicine and drug design

The great majority of drugs prescribed upon the classical paradigm of “one-drug-fits-all” and “trial-and-error” are effective only in 25-60% of the treated patients. Moreover, 50% from new chemical entities fail in the highly expensive phase III of clinical development. (Spraggs, 2009) Regulatory authorities FDA, European Medicines Agency (EMA) and the Japanese Pharmaceuticals and Medical Devices Agency (PMDA) recognize pharmacogenetics as an essential opportunity to predict drug responsiveness and to personalize therapy, and are increasingly integrating pharmacogenetic information to label updates of approved drugs, as well as incorporating pharmacogenetics into their regulatory review of new medicines. In addition, the regulatory framework that facilitates pharmacogenetics integration into drug development such as the Voluntary Exploratory Data Submissions in the USA and the Pharmacogenomics Briefing Meetings in Europe and Japan, as well as the more recent, formal biomarker qualification by the regulators are developed. (Surh et al., 2010)

5.1 Pharmacogenetic markers validation

Defining clinical guidelines for pharmacogenetic testing has to tackle the following issues: a) establishment of clinical end points; b) validation of pharmacogenetic testing in terms of its sensitivity, specificity, predictive power, cost-efficiency and time to perform; c) interpretation guidelines of the results and their impact on dosing algorithms, population’s stratification, clinical trials design, dissemination to clinicians and patients, incorporation into clinical practice without major interference to efficiency and cost of health care system. (Loo et al., 2010)

Pharmacogenetic algorithms should include clinical and genetic factors to guide therapy individualization, should be cost-effective and offer supplementary information over traditional approaches. However, broader dialogue and additional regulatory guidance are needed to reach a consensus regarding: the quantity of pharmacogenetic information in drug labels, indications for physicians (informative vs. recommended vs. required pharmacogenetic test) and measures to keep this information up to date, relevant sections on the labels (which currently ranges from Warnings to Indications to Clinical Pharmacology), levels of compelling evidence leading to decision-making translation (i.e., type of trial design, sample size, replication, reproducibility, consistency, effect size and other predictor variables). (Surh et al., 2010)

Regulatory recommendations for pharmacogenetic markers concern to: identification of responders and nonresponders; reduction of drug toxicity by minimizing or eliminating ADR; and optimization of the safety and effectiveness of drugs through personalized dosing strategies. This will enhance drug response predictability and safety in preclinical, clinical and postmarket trials. The biomarkers could be classified by their purpose in: improving drug safety, improving drug efficacy and confirming disease status. The FDA has three types of amendments for pharmacogenetic biomarkers, depending on the available evidence and the ability to implement the identified biomarker in clinical practice. These are as follows: information on the biomarker, which is strictly for informational purposes and does not require any action; recommended testing for the biomarker on the label; and mandated testing for the biomarker before drug use. (Squassina et al., 2010; Surh et al., 2010; Burns et al., 2010) Pharmacogenetic tests validated in clinical studies and recommended in the drug labels are detailed in table 1.
<table>
<thead>
<tr>
<th>Drug Indication</th>
<th>Pharmacogenetic biomarker</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mandatory, required predictive pharmacogenetic tests in drug label</strong></td>
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</tr>
<tr>
<td>Trastuzumab HERCEPTIN® Metastatic BC</td>
<td>HER2/neu over-expression</td>
<td>Improve drug efficacy: clinical benefit is limited to the responsive patients, whose tumors overexpress the drug-target HER2/neu (IHC or FISH assay)</td>
</tr>
<tr>
<td>Lapatinib TYKERB® Metastatic BC</td>
<td>HER2/neu over-expression</td>
<td>Improve drug efficacy: clinical benefit limited to tumors overexpressing HER2/neu (IHC or FISH assay)</td>
</tr>
<tr>
<td>Cetuximab ERBITUX® Metastatic CRC</td>
<td>EGFR expression</td>
<td>Improve drug efficacy: clinical benefit limited to patients with EGFR-positiv tumors (IHC assay)</td>
</tr>
<tr>
<td>Dasatinib SPRYCEL®, Imatinib GLEEVEC® ALL (adults)</td>
<td>Philadelphia chromosome positive</td>
<td>Disease confirmation and patients’ selection: BCR-ABL translocation (Philadelphia chromosome-positiv)</td>
</tr>
<tr>
<td>Maraviroc SELZENTRY® HIV (adults)</td>
<td>CCR-5 C-Cmotif receptor</td>
<td>Disease confirmation: infection with CCR-5-tropic HIV-1 and resistance to other antiretrovirals</td>
</tr>
<tr>
<td><strong>Recommended predictive pharmacogenetic tests in drug label</strong></td>
<td></td>
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<tr>
<td>Abacavir Ziagen® HIV infection</td>
<td>HLA-B*5701 allele</td>
<td>Improve drug safety: avoid hypersensitivity reactions to homozygous or heterozygous HLA-B<em>5701 genotypes. Screening is also recommended in reinitiation of drug in populations with previous tolerance of abacavir and unknown HLA-B</em>5701 status.</td>
</tr>
<tr>
<td>Azathioprine, IMURAN®, 6-MP PURINETHOL® ALL, inflammatory bowel disease</td>
<td>TPMT</td>
<td>Improve drug safety: avoid myelotoxicity in patients with phenotype or genotype of TPMT deficiency or lower activity. Subjects homozygous for TPMT*3A are at high risk for life-threatening myelosuppression when treated with standard doses of thiopurines: individualized doses are one tenth to one fifteenth the standard dose, in parallel with careful monitoring to avoid myelotoxicity. Patients with intermediate TPMT levels can safely receive thiopurines at lower doses (30–50% of the standard dose) and safe dose escalation under close monitoring.</td>
</tr>
<tr>
<td>Irinotecan CAMPTOSAR® CRC</td>
<td>UGT1A1</td>
<td>First FDA approved pharmacogenetic test “Third Wave Technologies, Invader assay” (2005), with dose optimization guidelines dependent on UGT1A1 genotype: avoid severe (grade III/IV) neutropenia and diarrhoea for those who are at high risk, i.e. homozygous (and possibly heterozygous) for UGT1A1<em>28 and UGT1A1</em>1 alleles.</td>
</tr>
<tr>
<td>Warfarin COUMADIN® Thrombo-embolism</td>
<td>CYP2C9 and VKORC1 (-1639G&gt;A)</td>
<td>Improve drug efficacy and safety: avoid increased risk of bleeding to patients homozygous or heterozygous for CYP2C9<em>2 or CYP2C9</em>3 alleles by prescribing differentiated doses (as compared with those for CYP2C9*1 homozygous). Pharmacogenetic test: “Nanosphere Verigene Warfarin Metabolism Nucleic Acid Test; therapeutic algorithm based on genotype and clinical factors (<a href="http://www.WarfarinDosing.org">http://www.WarfarinDosing.org</a>.)</td>
</tr>
<tr>
<td>Clopidogrel (prodrug) PLAVIX® Thrombo-embolism</td>
<td>CYP2C19</td>
<td>Improve efficacy and safety: doses adjustment for ultrarapid metabolizers who are carriers of CYP2C19<em>17/17 genotype and for poor metabolizers due to CYP2C19</em>2 allele presence.</td>
</tr>
<tr>
<td>Carbamazepine TEGRETOL® Epilepsy</td>
<td>HLA-B*1502 allele</td>
<td>Improve drug safety: avoid serious dermatologic reactions (Stevens-Johnson syndrome and/or toxic epidermal necrolysis).</td>
</tr>
<tr>
<td>Drug</td>
<td>Indication</td>
<td>Pharmacogenetic biomarker</td>
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<tr>
<td>Rasburicase</td>
<td>ELITEK&lt;sup&gt;®&lt;/sup&gt; Hyperuricemia</td>
<td>G6PD</td>
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<tr>
<td>Clozapine</td>
<td>CLOZARIL&lt;sup&gt;®&lt;/sup&gt; Schizophrenia</td>
<td>HLA-DQB1</td>
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<tr>
<td>Tretinoin</td>
<td>VESANOID&lt;sup&gt;®&lt;/sup&gt; APL</td>
<td>PML/RARα</td>
</tr>
<tr>
<td>Valproic acid</td>
<td>DEPAKENE&lt;sup&gt;®&lt;/sup&gt; Seizures</td>
<td>UCD deficiency</td>
</tr>
</tbody>
</table>

**Only informational pharmacogenetic tests in drug label**

| Panitumumab | VECTIBIX<sup>®</sup> Cetuximab ERBITUX<sup>®</sup> mCRC | K-RAS | Improve efficacy: clinical benefit limited to patients with non-mutated K-RAS. |
| Imatinib | GLEEVEC<sup>®</sup> GIST | C-KIT | Improve drug efficacy: clinical benefit in patients carriers of the activating C-KIT mutation |
| Busulfan | MYLERAN<sup>®</sup> CML | Philadelphia chromosome | Improve drug efficacy: responders are positives for Philadelphia chromosome (BCR-ABL) |
| Capecitabine | XELODA<sup>®</sup> CRC | DPD deficiency | Improve drug safety: decreased DPD and increased level of 5-fluorouracil is associated with severe toxicity (e.g., stomatitis, diarrhea, neutropenia and neurotoxicity). |
| Primaquine | Malaria | G6PD deficiency | Improve drug safety: avoid acute intravascular hemolytic reactions. |
| Isoniazid, Pyrazinamide | TB | NAT | Improve drug safety: dose adjustments based on NAT-metabolic status, for slow acetylators and fast acetylators to avoid severe adverse reaction of peripheral neuropathy, or lack of efficacy, respectively. |
| Erlotinib | TARCEVA<sup>®</sup> NSCLC | EGFR mutations | Confirm disease (at least 10% of the cells are EGFR-positive) and response to EGFR tyrosine kinase inhibitors |
| Lenalidomide | REVLIMID<sup>®</sup> Myelodysplastic syndromes | Deletion of chromosome 5q (del[5q]) | Confirm disease: indicated to treat those with transfusion dependent anemia caused by low- or intermediate-risk of myelodysplastic syndromes associated with 5q(del[5q]) |


Data taken from: Burns et al., 2010; Squassina et al., 2010; Loo et al., 2010; Spraggs et al., 2009; Sagreiya et al., 2010; Seip et al., 2010; Pare et al., 2010; Holmes et al., 2010; McDonald et al., 2009; Shuldiner et al., 2009; Mega et al., 2009.

Table 1. Predictive pharmacogenetic tests integrated into drug labels
5.2 Clinical trials with genotype-guided design

Pharmacogenetic cohort studies within randomized controlled clinical trials (RCT) may provide final evidence concerning the impact of specific genetic polymorphisms on the outcome of drug therapy. Patients are randomly assigned to groups receiving either the substance under investigation or the comparison (control) therapy. If pharmacogenetic genotypes are analyzed in all the participants in such studies, genotypes that differentially predict the response to the administered treatments may be identified. (Stingl & Brockmöller, 2011)

Adequate design and well-controlled clinical investigations with randomization elements are defined in the Code of Federal Regulations (CFR) (21CFR 314.126) and they provide the FDA with a legal framework for establishing evidentiary standards for approval of a new molecular entity, that is, they attest to a drug’s efficacy and safety when used under label conditions. In a pharmacogenetic context, RCTs can determine whether a genetic test, on average, is beneficial, harmful, or of no clinical value at the population level. When comparing the results of RCTs, there is a potential to find conflicting results due to differences in the null hypothesis, estimated effect size and study power, patient inclusion and exclusion criteria, clinical end points, and methods of data analysis. RCTs also have the potential to underestimate clinical events, for example, adverse reactions, when compared to “real-life” clinical events. (Lesko, 2007)

Although randomized controlled trials (RCT) are considered the gold standard for demonstrating the efficacy of therapeutic interventions, their application for the validation of many biomarkers, especially for multiple SNPs markers derived from GWAS, is limited by large sample sizes, time and financial restraints. Although pharmacogenetic studies as part of RCT allow to distinguish between prognostic markers and “true” predictive pharmacogenetic markers, there might be situations when RCT are unethical to conduct such as: the prescription of azathioprine or 6-mercaptopurine to homozygous carriers of thiopurine methyltransferase deficiency and the prescription of warfarin to combined carriers of low CYP2C9 activity and low vitamin K epoxide reductase complex subunit 1 (VKORC1) activity. (Lesko, 2007)

Alternatively, carefully designed retrospective and prospective case-control and cohort studies based on large, robust databases and conducted with appropriate power and corrections, will facilitate the discovery and replication of genotype-phenotype associations. Thus, prospective collection and banking of samples with appropriate consent, combined with retrospective DNA testing, is a necessity for exploration and potential validation of pharmacogenetic biomarkers. (Burns et al., 2010; Frueh, 2009; Stingl & Brockmöller, 2011) The case-control study design is the most frequently applied type of observational retrospective study in pharmacogenetics and genotype-related disease susceptibility research. The frequencies of genotypes in persons identified as having specific adverse drug events or poor therapy outcomes (“cases”) are compared with those of concurrently sampled controls who have had comparable drug exposure but who do not present the particular outcome. The advantages of case-control studies are: moderate resources, sufficiently high statistical power, performance in the natural settings of populations, focused on one type of well documented drug exposure. However, case-control studies are particularly prone to systematic error, also called “bias” and usually they have a supportive role requiring data replication in differently designed studies. (Stingl & Brockmöller, 2011)
The case–control Study of Hypersensitivity to Abacavir and Pharmacogenetic Evaluation (SHAPE) showed that 100% of both white and black patch test-positive patients carried HLA-B*5701, suggesting a 100% negative predictive value of HLA-B*5701 for abacavir HSR, generalizable across race. (Saag, 2010 as cited in Phillips et al., 2011)

A pharmacogenetic cohort study involves no controls and the group of patients receiving one type of therapeutic intervention is defined at the initiation of therapy and followed over the course of the study. This study design reflects true medical reality, is relatively easy to perform, and produced less biased results. In the time-series modified design, each patient serves as his or her own control because the drug treatment period is interrupted by a placebo period, thus a more precise differentiation between the drug effect and the effects of other factors may be obtained. The major drawbacks of cohort studies are: low statistical power requiring larger samples; a significant risk of confounding if there is heterogeneity in drug treatments, disease stages, and disease severity within the study sample; impossibility to differentiate between predictive and prognostic genotypes; insufficiency to serve as the only basis of therapy recommendations incorporating pharmacogenetics. (Lin & Chen, 2008 as cited in Stingl & Brockmoller, 2011)

Prospective interventional pharmacogenetic trials are based on the sequential pre-post or before-after design and patients receive drug therapies first before genotyping and then again after genotyping; sometimes it is also included a concurrently studied comparison group (parallel-controlled pharmacogenetic study) that implies randomized allocation to either the “therapy-guided by pharmacogenetic” arm or the “therapy as usual” arm. Prospective interventional pharmacogenetic trials allow a comparison between the efficacy of a new mode of treatment and that of the usual therapy and the extent to which the outcome of drug therapy would improve if it is guided by predictive pharmacogenetic testing. Randomized parallel diagnostic trials are more expensive, difficult to blind pharmacogenetic testing and might require larger sample sizes than phase III drug trials. (Haga et al., 2009 as cited in Stingl & Brockmöller, 2011)

Prospective on-going clinical trials with genotype-guided design may be illustrated by: a) IDANAT2 (“Isoniazid Dose Adjustment According to NAT2 Genotype”), initiated in Europe since 2008, aims to comparatively evaluate efficacy and liver toxicity of isoniazid administered in adjusted doses upon slow or fast acetylator status; b) phase II trial in North America on therapeutic strategy choice based on the presence of genotype tymidilate synthase TYMS TSER*3 “increased-risk” allele to the non-responders to fluoropyrimidines; c) phase III trial on rosiglitazone efficacy in Alzheimer’s patients stratified by APOE4 allele presence or absence; d) prospective study on Lapatinib (TYKERB) efficacy in metastatic breast cancer overexpressing Her2/ErbB2 to identify biomarkers of acquired chemoresistance to initial anthracyclins, taxans and trastuzumab therapy. (Spraggs et al., 2009)

PREDICT-1 is the first powered, randomized, blinded, prospective trial to evaluate the clinical utility of prospective pharmacogenetic screening for HLA-B*5701 to reduce the incidence of abacavir hypersensitivity in an abacavir-naïve population of HIV-infected subjects. (Hughes et al., 2008)

GENDEP is the largest prospective study to examine the interaction of a moderate effect size between genetic (SLC6A4 variants) and clinical predictors (stressful life event - SLE) on
response to antidepressants escitalopram and nortriptyline. The occurrence of antecedent SLEs predicted response to escitalopram and these effects were moderated by two functional polymorphisms 5-HTTLPR and STin4 in gene SLC6A4 encoding the serotonin transporter. (Keers et al., 2011)

The large, randomized controlled clinical trials COAG and EU-PACT (European Pharmacogenetics of Anticoagulant Therapy) will establish the safety and clinical utility of genotype-guided dosing in daily practice for the three main coumarin derivatives (warfarin, acenocoumarol, phenprocoumone) used in Europe, measuring as primary outcome the percentage time in the therapeutic range for international normalized ratio. (van Schie et al., 2009; Squasina et al., 2010) In the meantime, a new rapid and inexpensive Allele-Specific Amplification (ASA)-PCR genotyping assay for vitamin K antagonist pharmacogenetics was validated that may reduce the frequency of over- and undertreatment patients, especially during drug initiation, and thus will improve patient safety. (Spohn et al., 2011)

5.3 Pharmacogenetics in drug design and development

Pharmacogenetics is integrated in all phases of drug discovery and development. A) Preclinically, in high-throughput screening of whole genome expression profile in interaction with drugs and validation of new “druggable” targets; identification the ADME polymorphisms relevant to the investigational substance and evaluate ADME genotyping in all subsequent clinical studies; prediction of the risk of allergy and organ toxicities in carriers of specific genotypes before first application in humans. B) In phase I, identification and validation of pharmacogenetic biomarkers obtained from preclinical data in order to stratify patients into genotypic segments of responders vs. non-responders; guide phase II trials design; explain the lack of efficacy or adverse drug reactions, in more cost-effective manner than later, in separate human pharmacogenetic studies. C) In phase II, guide evidenced-based decisions about further development of the investigational substance; potential for further drug/genotype test co-development in phase III. D) Phase III, extensive biomarker research for clear evidence-based data concerning pharmacogenetics for drug labeling (genotype-defined subgroups having particularly high efficacy or high risk for ADR); improving risk–benefit ratio in the case of label extension; identification of innovative treatment principles and drug targets; enrollment in the phase III-IV clinical trials only of the group of patients highly predictable to respond to therapy and excluding those with high risk of adverse drug reactions according to genotype. E) Pharmacovigilance in the post-marketing phase of drugs allows optimization and label changes of approved drugs to include pharmacogenetic genotyping in order to exclude patients who carry genotypes predicting high risk ADR or no response. F) Reconsideration of potentially valuable drugs withdrawn because of adverse drug effects by excluding carriers of risk genotypes and by indicating only to genotypes predictive for high efficacy. (Stingl & Brockmöller, 2011)

Pipeline pharmacogenetics marks the change from the current lag phase towards the log phase, thus accelerating the rate of marketed new chemical entities, reducing the attrition rate during the expensive late phase clinical development, increasing the benefit to risk ratio through early identification of nonresponders or those individuals with high risk of ADRs, especially if the critical proof of concept for efficacy is prospectively predicted in the protocol for a clinical trial so as to be regarded by regulators as hypothesis testing. Pharmacogenetics has determined a paradigm shift within pharmaceutical industry towards
a “mini-blockbuster business model”, thus recognizing its significant commercial and success rate contribution. (Lesko, 2007) Among other pipeline pharmacogenetics’ benefits for pharmaceutical companies are: the opportunity to reconsider drugs which were initially stopped during development or withdrawn from the market; the ability to avoid investing in unfavorable products at earlier stages of development; the higher quality and more effective clinical trials design; reduction of research and development costs and periods; favorable impact on profit by increasing peak sales correspondent to a higher market share much earlier during the commercial life of the drug. For example, Eli Lilly has applied the tailored therapy strategy for drugs Xigris and Strattera. (Lechleiter, 2009, as cited in Fackler & McGuire, 2009; Roses, 2009)

Stingl and Brockmöller realize a comparative analysis of a variety of pharmacogenetics-related study designs integrated in all phases of drug development and clinical practice, evaluating the following issues: type and quality of evidence gained by each category of study design, their appropriated timeliness, as well as their pros and cons. The authors underline the necessity for new study designs for the clinical application of pharmacogenetics knowledge, as well as for mandatory requirements for the comprehensive characterization of relevant genetic variation in drug development. Future prospective interventional pharmacogenetic-trials in a genotype-preselected or outcomes-preselected population might represent one possible strategy to increase power in pharmacogenetic research at acceptable cost levels, especially to discover and validate the impact of genetic polymorphisms on drug response variability on the difficult-to-treat patients (poor-responders or severe adverse reactions) and in the elderly, on drug–drug interactions, or gene-gene interactions. (Stingl & Brockmöller, 2011)

Successful application of pipeline pharmacogenetics should meet some basic requirements, such as: obtaining the appropriately-documented informed consent; collection and storage of DNA samples for regulatory submission; implementing predictive exploratory pharmacogenetics studies on candidate gene and pathway-associated SNPs (rather than GWAS) during phase I clinical trials to generate hypothesis for early phase IIA, followed by hypothesis testing during phase IIB, in order to increase the statistical significance within a relatively smaller, well-defined group of patients. However, standardized methodologies should be established regarding: specification of sample source, standardization of diagnostic systems and treatments, adequate monitoring, sufficient length of observation, inclusion of possible confounding factors. (Roses, 2009; Spraggs et al., 2009)

The cost of pipeline pharmacogenetics is much lower in comparison with the cost of clinical trials and drug attrition. The advantages of large public-private partnerships at all stages of drug development in order to accelerate new medicines approval and biomarkers qualification and validation for selection of patients for clinical trials, monitoring drug effects and safety risk, regulatory guidelines harmonization and implementation, are illustrated by Eck and Paul. (Eck & Paul, 2010)

6. Pharmacogenetics’ perspectives and challenges

High priority directions in the pharmacogenetic research for maximizing its potential benefit for personalized medicine are: 1) Chronic diseases (hypertension, diabetes, asthma, epilepsy, multiple sclerosis etc) which imply: high costs for healthcare system and patients,
considerable management care costs; polymedication; low clinical outcomes due to compromised patients’ quality of life, continuous suffering and low compliance to medication. 2) Medications with long adjustment periods of therapeutic doses and currently prescribed on “trial-and-error” basis, resulting in reduced compliance rates and increased management care costs. 3) Drugs with narrow therapeutic index (mercaptopurine, warfarin) that require close monitoring and constant dosage adjustment procedures to maintain therapeutic efficacy and minimize adverse reactions. 4) Drugs with severe, life-threatening adverse reactions which require high hospitalization costs (abacavir). 5) Very expensive medications with high clinical efficacy in some segments of population and for which a predictive diagnostic test might be developed (herceptin). (Grossman & Goldstein, 2009)

Pharmacogenetic testing is already implemented in some clinical areas, such as in cardiovascular diseases or in cancer, for selecting and/or dosing a specific medication, while in other fields, such as in psychiatry, the pharmacogenetic approach has been mostly used for the identification, validation and development of new meaningful biomarkers. (Squassina et al., 2010)

Of particular interest is pediatric pharmacogenetics that should consider both variation in gene expression and developmental context in which the genes of interest are functionally active (ontogeny). Apart from the application of pharmacogenetic testing to children for thiopurine-induced myelotoxicity, no other genotype-drug response associations validated in adults have been conclusively validated and no diagnostic or pharmacogenetic dosing algorithms have been so far translated in pediatric patients. (Ross CJ et al., 2011; Becker & Leeder, 2010) In order to achieve this gap, the Canadian Pharmacogenomics Network for Drug Safety (CPNDS) has implemented an active and targeted adverse drug reaction surveillance in pediatric patients with the aim of functional validation of identified gene markers, such as: warfarin-induced bleeding and thrombosis, vincristine-induced peripheral neuropathy, glucocorticoid-induced osteotoxicity, methotrexate-induced mucositis or leukoencephalopathy or nausea and vomiting, anthracycline cardiotoxicity, cisplatin-induced ototoxicity, neurotoxicity and nephrotoxicity. The final objective is to ensure generalizability of clinically significant findings and translation into a pharmacogenetic test of each predictive biomarker evaluated in prospective clinical trials (such as those dedicated to cisplatin ototoxicity and codeine-induced mortality in breastfed infants by mothers who are CYP2D6 ultrarapid metabolizers and UGT2B7*2 homozygous carriers), as well as to provide a cost–benefit analysis for validated predictive biomarker. (Loo et al., 2010)

6.1 Translational challenges

Successful integration of pharmacogenetics into future clinical practice and personalized medicine should overcome further translation barriers related to: a) global problems of scarcity of data demonstrating pharmacogenetic testing’s clinical validity and utility; b) standardized and powerful statistical correlations between genotype and drug-response phenotype across different populations; c) education of health professionals and public; d) pharmacoeconomics issues; e) bioethical and social aspects; f) more favorable regulatory policy for clinical uptake of pharmacogenetics via sustaining large-scale industry-academia collaborations; g) the adoption of harmonized and standardized guidelines for biorepository and data sharing across multi-national networks; h) lack of incentives for the private sector
to invest in the development and licensing of pharmacogenetics diagnostic tests for improving the safety and efficacy of out-of-patent drugs. (Spraggs et al., 2009; Gurwitz et al., 2009)

Scientific community and International Society of Pharmacogenomics Education Forum have called for the enhanced implementation of pharmacogenomics and personalized medicine into core medical teaching curricula and practice, in order to fill in the gap between intensive research on validated pharmacogenetic testing and its appropriate, effective clinical integration and interpretation in routine individualized therapy. (Squassina et al., 2010) For instance, Pharmacogenomics Education Program (PharmGenEd™) is an educational platform for dissemination of evidence-based clinically relevant pharmacogenomics, fostering educational and scientific collaboration among educators, researchers and clinicians (http://pharmacogenomics.ucsd.edu). PharmGenEd also participates along with Pharmacogenomics Knowledge Base (PharmGKB) and National Institute of Health Pharmacogenomic Research Network (NIH PGRN), in the Clinical Pharmacogenetics Implementation Consortium (CPIC) to create a curated resource for storing, annotating and updating specific data relevant for clinical implementation of pharmacogenetic testing. (Kuo et al., 2011)

The adoption of the pharmacogenetics tests for personalized medicine depends on clinical- and cost-effectiveness analyses on which the reimbursement for their routine use will be based. (Liewei et al., 2011) In the context of pharmacoeconomic models, pharmacogenetic testing might be regarded more cost-effective than cost-saving, or at least cost-effective for particular combinations of treatment, genetic polymorphisms and disease, depending greatly on the differences among healthcare systems and reimbursement policies. (Squassina et al., 2010; Trent, 2010)

Appropriate protection for privacy and confidentiality of databases with large amount of genotypic, phenotypic and demographic data regarding individuals is crucial in order to avoid the possible risk of psychosocial harm, genetic and social discrimination, privacy and possible implications for employment and access to life and health insurance. (Squassina et al., 2010) Moreover, pharmaceutical companies could voluntarily ignore, for economic reasons, patients with rare or complex genetic conditions or those who are not responding to any known treatment, leading to consequent deprivation of effective treatments. These concerns are attenuated by USA specific regulations, such as the Genetic Information Nondiscrimination Act (GINA), the Health Insurance Portability and Accountability Act Privacy Rule, and the Genomics and Personalized Medicine Act (GPMA), as well as by founded nonprofit organizations of major key stakeholders (pharmaceutical companies, healthcare providers and payers, patient advocacy groups, industry policy organizations, academic institutions and government agencies), like the Personalized Medicine Coalition and Pharmacogenetics for Every Nation Initiative (PGENI). (Squassina et al., 2010)

Taking into account the ethical, legal and social issues of pharmacogenetics research, as well as the multi-disciplinary opinions of the key stakeholders, Howard and colleagues have identified six outstanding ethical issues raised by the informed consent process in pharmacogenetics research and proposed valuable recommendations for the development of future practical pharmacogenetics consent guidelines, such as: 1) scope of consent; 2) consent to pharmacogenetics ‘add-on’ studies; (3) confidentiality, privacy protection and
Pharmacogenetics: Matching the Right Foundation at Personalized Medicine in the Right Genomic Era

Coding of personal information in long-term databases, especially in those controlled by private pharmaceutical companies; (4) intellectual property and commercialization policy of pharmacogenetics research projects with financial benefit-sharing plan; (5) disclosure of the possibility of deposition and sharing in public repository of the samples and data, at the onset of the pharmacogenetics study; (6) potential risks stemming from population-based research so as to avoid overgeneralizations and undermining the moral, cultural and religious standards. (Howard et al., 2011)

6.2 Pharmacogenetics’ perspectives by next generation sequencing

GWAS are particularly useful to determine the most significant SNPs associated with a phenotype amongst a high-density set of polymorphisms, in cases where there are large cohorts to evaluate, clinical end points are simple to define without variability, and the genetic factors associated with the end points are highly penetrant. However, GWAS are discovery- rather than hypothesis-driven, result in weak statistical power and false positives, need large sample size, cost and computing power requirements. Therefore, GWAS imply a two-stage design where discoveries made using a high-density SNP array are then validated using a hypothesis design on replicative populations. (Sissung et al., 2010) Nonetheless, for ADR with a significant genetic component that confers a large effect (carbamazepine-induced Stevens–Johnson syndrome, abacavir-induced hypersensitivity, statin-induced myopathy and gefitinib-induced diarrhoea), associations can be identified with a relatively small number of samples (10–100 cases). (Loo et al., 2010; Davis & Johnson, 2011)

Rare variants in complex mixtures of DNA might be quantitatively measured by mass spectrometry-based genotyping. Fluidigm’s microfluidic device (“lab-on-chip”), able to mix 96 samples and 96 primer sets in nano-scale assay chambers that support over 9000 parallel qRT-PCR reactions, could be also used for genotyping and targeted sequence capture; thus filling the gap between biomarker evaluation of a few candidate genes and the hundreds of markers GWAS-discovered. (Gibson, 2011)

The development of next-generation sequencing (NGS) or massively parallel sequencing, as well as nanopore sequencing technologies, through whole-genome, whole-exome and whole-transcriptome analysis, allows fast, inexpensive, reliable production of large volumes of DNA or RNA sequence data. (Metzker, 2011; Tucker et al., 2009) NGS comprises a number of methods that are grouped broadly as template preparation, sequencing and imaging, genome alignment and assembly, and data analysis. The unique combination of specific protocols distinguishes one technology from another and determines the type of data produced from commercial platforms such as: Roche/454, Illumina/Solexa, Life/APG and Helicos BioSciences, the Polonator instrument and the Pacific Biosciences. The NGS technologies, their broad applications and guidelines for platform selection to address biological questions of interest are reviewed by Metzker (Metzker, 2011). Having aligned the digested fragments of individuals’ targeted regions of genome to a reference genome, ‘SNP calling’ identifies variable sites, whereas ‘genotype calling’ determines the genotype for each individual at each site, thus revealing principal types of genome alterations (like nucleotide substitutions, small insertions and deletions, copy number alterations, chromosomal rearrangements). (Rasmus et al., 2011) Computational, biological and clinical analyses of the resulting genome data will assess reproducibility and statistical significance; links to
pathways and the functional relevance of mutated genes to disease; and the relationships of genome alterations with cancer prognosis and response to therapy, respectively. (Meyerson et al., 2010)

Over a relatively short time frame, DNA sequencing has become cheaper and faster (US$ 1,000 price tag for a whole human genome sequence now seems feasible) and even more with the foreseeable third-generation DNA sequencers utilizing single molecules and avoiding initial cloning or amplification steps. (Trent, 2010; Pushkarev et al., 2009)

In addition to NGS rapid technological developments, the promotion of alternative strategies for delivery of healthcare through nontraditional pathology laboratories, such as direct-to-consumer (DTC) DNA tests and point-of-care DNA testing, will expand the integration of pharmacogenetics into clinical practice by assisting health practitioner to make on-the-spot decisions about the individualisation of drug and dose directly at the bedside in the intensive care unit or consulting room. (Trent, 2010)

6.3 New players in pharmacogenetics: Alternative splicing, miRNAs and structural RNA polymorphisms

In addition to polymorphisms in protein-coding genes (nonsynonymous SNPs) associated to complex diseases, alternative splicing in protein-coding genes and variations in microRNAs and other noncoding RNAs have emerged as new players in pharmacogenetics and they should be considered in an integrative approach in deciphering the genotype-induced inter-individual variability of drugs’ tolerance, efficacy and metabolism.

Alternative splicing of pre-mRNAs was proposed 30 years ago by the Nobel Prize winner Walter Gilbert as a way of generating different mRNAs from a single gene and it is regarded as one of the most elegant and important mechanisms for proteome diversity generation. Alternative splicing in protein-coding genes can affect the biological activity of proteins, having major consequences on drug metabolism and drug response phenotype. Generation of wrong alternative splicing variants is a common feature of complex diseases and also an important player in drug resistance and ADME. For example, alternative splicing variants in the BCR-ABL fused gene were correlated with Imatinib mesylate resistance in chronic myelogenous leukemia patients (Gruber et al., 2006, as cited in Passetti et al., 2009); whereas splicing isoforms of nuclear pregnane and constitutive androstane receptors can affect the pharmacokinetics and pharmacodynamics of docetaxel and doxorubicin in Asian patients. (Horr et al., 2008, as cited in Passetti et al., 2009) The functional relevance of alternative splicing of pre-mRNAs and generated splice variants in the context of whole genome studies instead of a single gene would reveal how they will affect cellular networks and pathways. (Passetti et al., 2009)

The noncoding RNAs (ncRNAs) are a large group of transcripts lacking protein-coding potential, having variable size range from approx. 18 to 25 nucleotides for the families of microRNAs (miRNAs) and small interfering RNAs (siRNAs), approx. 20 to 300 nucleotides for small RNAs commonly found as transcriptional and translational regulators, or up to and beyond 10 000 nucleotides in length for RNAs involved in various other processes. (Costa FF, 2008, as cited in Passetti et al., 2009) miRNAs are noncoding RNAs that can regulate gene expression by Watson–Crick base pairing to target several mRNAs in a gene
regulatory network. The binding of miRNAs to their target mRNAs is critical for regulating mRNA levels and therefore protein expression. miRNAs are master regulators of important gene and transcriptome networks in eukaryotic cells, they can block mRNA translation and affect both the expression of at least 30% of all protein-coding genes by targeting their 3'-UTR sequences and long ncRNAs. A growing number of reports have been showing the associations of deregulated expression of miRNAs in complex diseases (cancer, obesity, diabetes, schizophrenia) by altering the regulation of expression of many important genes. One miRNA can downregulate multiple target proteins by interacting with different target mRNAs ('one hit multiple targets' concept), thus pointing out the particular therapeutic relevance of miRNAs as an attractive drug target. (Wurdinger & Costa, 2007, as cited in Passetti et al., 2009) Moreover, polymorphisms in miRNAs represent a newly identified type of genetic variability that can influence the risk of diseases and also variability in pharmacokinetics and pharmacodynamics of drugs. For instance, polymorphisms in miRNA target sites of protein-coding genes are associated to cancer, hypertension, asthma, cardiovascular disease, and polymorphisms in microRNAs are associated with schizophrenia, Parkinson. (Passetti et al., 2009) In addition, long ncRNAs, increasingly seen as functional genes, have been involved in disease progression, such as: ZNFX1-NA1 in breast cancer, SPRY4-IN in melanoma; NEAT1 long ncRNA in Alzheimer. (Gibson, 2011)

Alternative splicing might affect microRNA regulation and subsequently microRNAs are able to regulate hundreds of effector genes in a multilevel regulatory mechanism that allow individual miRNAs to profoundly affect the gene expression program in the cells. Both microRNA regulation and alternative splicing will induce changes in proteome diversity that can affect the way drugs are metabolized by patients, and this will have major implications for both drug design and personalized medicine in the future. Furthermore, genetic variations in the sequence of miRNAs, target sites of miRNAs and alternative splicing will affect gene regulatory networks and pathways responsible for drug metabolism and resistance, thus emerging as a new paradigm clearly redefining the pharmacogenomics field and personalized medicine. (Passetti et al., 2009)

Structural RNA SNPs (srSNPs) designate genetic polymorphisms in the transcribed regions of genes that affect RNA functions and represent ~49% (synonymous SNPs, 2%; those in 5'- and 3'-untranslated regions, 2%; intronic, 45%) from disease-associated SNPs derived from GWAS, whereas nonsynonymous (coding cSNPs) account for only ~9% and regulatory SNPs (rSNPs; SNPs in intergenic regions that alter transcription of protein-coding genes) for ~43%. (Hindorff et al., 2009, as cited in Sadee et al., 2011) Structural RNA SNPs affect RNA functions such as splicing, turnover and translation, having tissue-specific effects, while regulatory single-nucleotide polymorphisms (rSNPs) affect transcription. (Sadee et al., 2011)

Recent GWAS and next-generation sequencing of the transcriptome have opened path for large-scale exploration of mRNA expression quantitative trait loci (eQTLs) which are commonly categorized as rSNPs affecting transcription and altering mRNA expression levels in target tissues. (Sadee, 2009, as cited in Sadee et al., 2011) Furthermore, the evaluation of allelic ratios of transcripts would enable the detection of rSNPs and srSNPs, by revealing any deviation from unity expected in an autosomal gene - termed “allelic expression imbalance” (AEI) - that indicates the presence of cis-regulatory factors and represents a more precise relative measure of transcript activity as compared with total mRNA levels. (Johnson, 2008, as cited in Sadee et al., 2011) Structural RNA polymorphisms
will likely prove essential to fill gaps in the “missing heritability”, substantially contributing to discover pharmacogenetic biomarkers of increased predictive power. The clinical relevance of rSNPs and srSNPs has greatly contributed the available validated predictive genetic variants. For example, there are validated srSNPs and rSNPs in DME (CYP2C19, CYP2D6, CYP3A5, UGT1A1, NAT1, ABCB1, ABCC2) associated with variability in the bioactivation, pharmacokinetics and clinical outcome of clopidogrel, tamoxifen, statins, efavirenz, tacrolimus, paclitaxel, antiretrovirals. Moreover, validated srSNPs and rSNPs in genes that encode drug targets (D2 dopamine receptor gene DRD2) are associated with poor response to antipsychotics or with risk for metabolic syndrome (rSNPs in gene TPH2 encoding tryptophan hydroxylase). (Sadee et al., 2011)

6.4 Pharmacogenetics' perspectives by biobanking and electronic health records

Global surveys conducted by the Industry Pharmacogenomics Working Group (I-PWG) in order to determine current industry and institutional review boards/ ethical committee (IRB/EC) practices, policies and standards, for prospective biospecimens’ collection and storage for pharmacogenomics research, emphasize the significant value of pharmacogenetics research and biobanking for personalized medicine, as well as the necessity for harmonization and standardization across the industry and the key stakeholders in regulations concerning: sample acquisition and data privacy protection, pharmacogenetics-related language in informed consent, outsourcing of DNA sample storage, “clinical relevance” of the genetic information to be returned to the patients, benefits and foreseeable risks. (Franc et al., 2011a, 2011b; Warner et al., 2011; Ricci et al., 2011)

The creation of a biorepository that are closely link with electronic medical records (EMR) may be an economically efficient approach to genomic medicine and especially to GWAS that require DNA sample from large populations with robust phenotypic data. The Geisinger MyCode Biorepository is perhaps the first large-scale biobanking project around EMR. In addition, a data warehouse project – the Clinical Decision Information System – initiated in 2006 aims to assembly in a single point of reference 40 different data sources regarding patients’ clinical phenotype, biobanks, clinical trials databases, as well as financial, administrative, operational and patient survey databases. Such data warehouses allow optimizing performance, maintaining control and privacy aspects, reliability, robustness and fast-response. (Gerhard et al., 2009)

Large multi-national consortia have been already established, such as NIH-funded PGRN, and NIH-funded Electronic Medical Records and Genomics Network (eMERGE), which are catalogued in Pharmacogenomics Knowledge Base. (McCarty & Wilke, 2010)

The convergence of the rapidly expanding biomedical informatics, high-throughput genotyping, DNA biobanks and EMR across large health-care networks, plays a pivotal role in pharmacogenomics’ translation to the bedside. Through real-time monitoring of multiple de-identified EMR databases integrated in sophisticated cross-institutional networks (e.g., the PGRN, the eMERGE network, and the HMO Research Network, Harvard University/Partners Healthcare system i2b2, the Vanderbilt BioVu), highly accurate quantifying disease phenotypes and treatment outcomes could be efficiently extracted by the application of natural language processing (NLP), semantic interoperability, data normalization strategies and novel bioinformatics platforms. The following translational
mechanisms are envisaged: (i) retrospective assessment of previously known findings in a clinical practice-setting; (ii) discovery of new associations in huge observational cohorts; (iii) prospective application in a setting capable of providing real-time decision support; iv) enhance pharmacovigilance, especially during the postmarketing phase; vi) validation of previous conventional cohort-driven GWAS, in a cost-effective, earlier and more accurately manner. (Wilke et al., 2011; Kohane, 2011) In addition, electronic health records-driven genomic research (EDGR) will provide a rich set of comprehensive clinical phenotypes, in close to real time, at a low cost and a high degree of timeliness, matched to the corresponding DNA samples from biorepositories. The cost-efficiency advantage of EDGR comes from maximizing the research utility of that clinical-care investment such that it is only a fraction of a de novo research cohort pipeline. Other advantages of EDGR include: ability to assess in-depth the clinical significance of genomic associations; great representation of a clinical population; data on environmental exposures; broad and accurate reflection of clinical phenotypes and controls; identification of confounders. (Kohane, 2011) In the future, as standardization across national biobanks-linked EMR, consent procedures and ownership of the derived intellectual property will be adopted, genetic data will be recorded preemptively into each patient’s EMR, and robust biomedical informatics platforms will interrogate this information during the process of clinical decision making, providing efficient real-time decision support at the point of care. (Wilke et al., 2011; Kohane, 2011)

Development of simple, up-to-date, easily accessible, reliable clinical algorithms and guidelines must guide physicians in the interpretation of genetic data, decision making about diagnostic testing and follow-up clinical care. These point-of-care tools will be embedded in electronic health records system and it will be crucial to accelerate the individualized medicine. (Fackler & McGuire, 2009; Liewei et al., 2011)

In addition, the implementation of robust health information technology able to electronically manage all different types of -omics biomarker data and phenotypic characterization of research study participants, might be illustrated by software platforms like: Hewlett-Packard’s Gateway for Integrated Genomics - Proteomics Applications and Data (GIGPAD) or Microsoft’s Amalga. (Fackler & McGuire, 2009)

The National Institute of Health’s Pharmacogenomics Research Network (PGRN) is a collaborative partnership of research groups funded “to lead discovery and advance translation in genomics in order to enable safer and more effective drug therapies”, with the ultimate goal to predict and personalize medicine in routine clinical practice. (Long & Berg, 2011) PGRN’s accomplishments and future projects to provide peer-reviewed, updated, evidence-based, freely accessible guidelines for gene/drug pairs, so as to facilitate the translation and interpretation of preemptive pharmacogenomic tests for the most relevant pharmacogenes from laboratory, through electronic medical records system, into decision-making prescription recommendations, have been extensively discussed. (Long & Berg, 2011; Roden & Tyndale, 2011; Relling & Klein, 2011)

6.5 Further perspectives in pharmacogenomics

Further perspectives for pharmacogenomics to emerge as an important piece in the puzzle of personalized medicine will concern: a) the integration of pharmacogenomics with
additional, non-drug-related patient characteristics, individual disease factors, and environmental aspects (Kroemer, 2010); b) tissue-specific epigenetic changes, microRNAs or “junk DNA” (Trent, 2010); c) discovery of useful pharmacogenetic markers in the mitochondrial DNA (mtDNA) within transmitochondrial cell lines or cybrids in order to optimize antibiotic therapy (Squassina et al., 2010); d) biomarkers validation for drug therapy in organ transplantation and for complex diseases characterized by a great phenotypic and genetic variability; e) coordinated implementation into certified laboratories of the pharmacogenes’ next-generation-sequencing, according to GCP guidelines; f) the adaptation of the regulatory and reimbursement environment. (Fackler & McGuire, 2009)

Moreover, nanotechnology is projected to play a critical role in personalized medicine, greatly dependent on the evolutionary development of a systems biology approach to clinical medicine based upon “-omic” technology analysis and integration. In a comprehensive review, Sakamoto and colleagues analyse: the current state of nano-based products over a vast array of clinical indications and patient specificity; rational design of nanotechnologies for individualized therapy; nano-based injectable therapeutics, implantable drug-delivery devices; nanotechnology and tissue engineering; nanowires and cantilevers arrays that are used to detect minute amount of protein biomarkers. (Sakamoto et al., 2010)

7. Conclusions
Pharmacogenomics is the interface between genomic medicine and systems pharmacology and its translation from bench into clinical practice is broadening the perspective of personalized medicine so as in the near future we might rely on a “DNA chip”/“pharmacogenomic card” specific to each patient and on each genotype preemptively recorded in EMR, in order to individualize both the diagnostic procedures and the safest and most efficient medications prior to treatment initiation. Although it is still regarded as an elusive dream due to limited marketed drug–test companion products and actually implemented clinical practices, pharmacogenomics translation into personalized medicine has become a more imminent reality. Advances in next-generation sequencing technology to uncover the contribution of “missing heritability” to biomarker-guided therapeutic individualization, the convergence of biorepositories- and electronic health records-pharmacogenomics research, the encouraging initiatives for policy and guidelines harmonization, as well as extensive collaborations across large pharmacogenomic networks, will hopefully overcome the current challenges ahead on the road to personalized medicine and will play a pivotal role in pharmacogenomics’ translation to the bedside.

8. References
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The rapidly evolving field of Pharmacogenetics aims at identifying the genetic factors implicated in the inter-individual variation of drug response. These factors could enable patient sub-classification based on their treatment needs thus expediting drug development and promoting personalized, safer and more effective treatments. This book presents Pharmacogenetic examples from a broad spectrum of different drugs, for different diseases, which are representative of different stages of evaluation or application. It has been designed so as to serve both the unfamiliar reader through explanations of basic Pharmacogenetic concepts, the clinician with presentation of the latest developments and international guidelines, and the research scientist with examples of Pharmacogenetic applications, discussions on the limitations and an outlook on the new scientific trends in this field.

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