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REVIEW ARTICLE

Pharmacogenomics: Current applications and future prospects towards personalized therapeutics

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Summary

Personalized Medicine is more than just a metabolic activity of a person. Pharmacogenomics, pharmacogenetics, pharmacoproteomics, and metabolomics play an important role in the development of personalized medicines. Personalized medicine uses information about a person's genes, proteins, enzyme activities, and cellular environment to diagnose and treat disease, cancer included. A major problem of personalized medicine is the fact that there is no portable bedside and low-cost bioanalytical technology that can be used in close proximity to the patient. This technology could play a significant role in defining the dosage setting for subsets of the population. The success of the personalized therapy is possible through the application of technology, which can provide a bridge between metabolism status and an individual's response to a particular drug and therapeutic modality.

Key words: *cancer, epigenetics, personalized medicine, pharmacogenomics, polymorphisms*

Introduction

Over the past decade, the technology of studying genomes has made considerable improvements which are enabling medicine to reveal a specific patient's genome in order to design a personalized therapy.

Pharmaceuticals from a wide spectrum of therapeutic classes are used in human medicine worldwide and include more than 4000 molecules with different physicochemical and biological properties and distinct modes of biochemical action. After administration of the active pharmaceutical compounds, some drugs are metabolized, while others remain intact before being excreted [1].

Pharmacogenomics is the study of variations in DNA sequence related to drug response. Common genetic variations are single-nucleotide polymorphisms (SNPs), genetic insertions and deletions, and genetic copy-number variations (CNVs). Both SNPs and CNVs play a role in pharmacogenomics, in different phenotypic outcomes and measures. More specifically, pharmacogenomics studies genetic variations in enzymes metabolizing drug, receptors, transporters, and targets, and how these variations interact to produce effects such as drug response or toxicity. It has also identified the causes of inter-individual variations in the expression and function of many of these genes, including the role of microRNA (miRNA), DNA methylation, copy-number alterations, and single-nucleotide differences, either inherited SNPs or somatically acquired single-nucleotide variants [2,3]. More than 800 medicinal agents have been developed to target specific genetic mutations.

These epigenetic mechanisms manifest mostly through changes in chromatin packing and in localized gene promoter changes that influence the transcription of the genes involved in carcinogenesis [4].

Pharmacogenetics, on the other hand, informs development of safer prescribing criteria and more effective drugs. Furthermore, genetic markers can indicate novel drug targets or modifiers, serving to functionally classify the disease and thereby influence the design of the treatment. Personalized Medicine, based on scanning a person's genome,

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will reveal genetic risks for developing various diseases and their genetically governed reactions to specific medication [5]. It may also potentially benefit on medicine including minimizing risk of drug toxicity, increasing benefit from drugs used, contributing to the sustainability of healthcare system and facilitating drug discovery and development programs [6].

During the past decades, cancer research provided great knowledge in genomic alterations involved in tumor development. These genomic changes may influence genes encoding drug-metabolizing enzymes or drug transporters, leading to alterations in the disposition of active drugs at the site of the tumor [7]. For instance, this knowledge helped the development of targeted cancer therapy in Philadelphia chromosome-positive chronic myeloid leukemia or acute lymphoblastic leukemia, targeting the BCR/ABL1 fusion transcript.

Currently, prescribing on the basis of population data does not guarantee benefit for the individual. Prescribing by genotype offers the patient the potential benefit that physicians will prescribe the right drug at the right dose. This approach maximizes efficacy and minimizes toxicity. Pharmacogenetic tests offer improvement in short-term measures and in long-term mortality. Despite the potential benefits, there are other aspects of relevance to patients that have to be considered. First, given that these are going to be DNA-based tests, specific safeguards to maintain confidentiality will have to be put into place. Laboratories carrying out such testing will need to undergo an accreditation process to ensure safe and secure storage of both samples and information. However, if pharmacogenetic information leads to prescription of a particular drug, the mere fact that the patient is on the drug will betray their genotype, even without direct knowledge of the results of their genetic test [8,9].

Pharmacogenetic tests may also have implications for family members. Genetic tests may disclose non-paternity, particularly when other family members have been tested. The pharmacogenetic test may also indicate an increased predisposition to developing certain adverse effects. It may be necessary to undertake family screening, as is currently practised for probabilistic tests such as the Factor V Leiden mutation.

In future, pharmacogenomics will increase understanding of the genetic basis of drug response and help develop more effective and less toxic treatment for individual patients.

The aim of this short review article was to provide an overview on recent pharmacogenomic approaches regarding current applications and future prospects towards personalized medicine, including targeted cancer monoclonal therapies [10].

Pharmacogenomics in current therapeutics

Drug development is a really expensive process, costing at approximately €500-800 million per marketed drug. It also takes about 10-15 years for each drug to reach the market after discovery [8]. In addition, there is a high attrition rate with only one out of every 5000 or even 10000 chemical compounds considered to have a therapeutic potential being successfully developed for clinical use. The incorporation of pharmacogenomics into the drug development process has the potential to improve target identification, accelerate the development process and reduce the attrition rate.

Pharmaceutical companies and health care systems have to consider the variability in the way individuals respond to drugs, in terms of both efficacy and toxicity [11]. For example, there is a 20-fold variation in the dose of *warfarin* required to achieve optimal anticoagulation across patients. Adverse drug reactions are also a major problem, costing 5% of all hospital admissions and increasing the length of stay in hospital by 2 days [12]. Predisposing to adverse drug reactions correlates with polymorphisms in drug metabolizing enzyme genes [13]. A large number of drugs cited in adverse drug reactions are metabolized by at least 1 enzyme with a variant allele associated with reduced activity. Also, adverse drug reactions are likely to have more than one genetic predisposing factor.

Additionally, adverse reactions and efficacy are invariably the outcome of both genetic and non-genetic factors. The potential benefits, in both health and economic terms, are considerable. However, therapeutic intervention based on individuals' genetic variation will not be applicable to all drugs and careful evaluation of cost effectiveness will be needed on case-by-case basis [13,14].

Pharmacogenetics into clinical practice has the potential to improve efficacy and reduce toxicity, by choosing "the right drug for the right patient in the right disease at the right dose".

Pharmacogenomics -Drug metabolism and development

Proteomic and genomic technologies may increase the diversity of targets available for future

medicinal agents by identifying novel proteins, targeting proteins with variant structure, identifying mechanisms of action of drugs, developing compounds and increasing the specificity of drug action.

Pharmaceutical metabolism taking place in several human organs, mostly the liver, is divided into three phases. In phase I, enzymes such as cytochrome P450 oxidases introduce reactive or polar groups into the pharmaceutical compound.

These enzyme complexes are due to incorporate an atom of oxygen into non-activated hydrocarbons, which can result in either the introduction of hydroxyl groups or N-, O- and S-dealkylation of drugs. The reaction mechanism of the P450 oxidases proceeds through the reduction of cytochrome-bound oxygen and the generation of a highly-reactive oxyferryl species [15,16].

Phase I chemical reactions may occur often in the liver by oxidation, reduction, hydrolysis, cyclization, decyclization, and addition of oxygen or removal of hydrogen, carried out by oxidases.

The modified compounds are then conjugated to polar compounds in phase II reactions. The above reactions are catalyzed by transferase enzymes such as glutathione S-transferases. In phase III, the conjugated drugs may be further processed, before being recognized by efflux transporters and pumped out of cells [17].

On the other hand, clinical trials involving new drugs are commonly classified into four also distinct and different phases. The drug development process will normally proceed through all four phases over many years. If the drug successfully passes through phases I, II, and III, it will usually be approved by the national regulatory authority for use in the general population.

Pharmacogenetics may lead to refinement of phase I studies by focusing on individuals with known genotypes defined through preclinical testing. An earlier identification of problems may lead to the compound being dropped during phase I rather than in phase III, with considerable savings in development costs. In phase II, there may be further refinement of the pharmacogenetic determinants of drug response, which may provide information necessary for design of the phase III studies. The sample size for phase III studies may be reduced, but there is a possibility that more individuals will need to be studied during phases I and II [18]. The expected benefits of pharmacogenetic applications in clinical trials are summarized in Table 1.

In the meantime, pharmaco-epidemiological studies take place and can continue for the whole period the drug is on the market. Phase IV allows the detection of rare adverse events occurring in this phase. Samples from patients treated with the drug can be used for pharmacogenetic testing and identification of genetic predisposing factors, allowing an improvement in the risk-benefit ratio [18].

Table 1. Pharmacogenetics in clinical trials

Clinical trials	*Pharmacogenetics*
Phase I	Detection of individuals with known genotypes
Phase II	Refinement of the pharmacogenetic determinants
Phase III	Reduction of studies, or re-evaluation of phases I,II
Phase IV	Drug is licenced

Biomarkers in pharmacogenomics/ genetic variations and drug response

SNPs-CYPs

Childhood acute lymphoblastic leukemia (ALL) is the classic example for a drug-responsive malignancy, and contemporary risk-directed therapies cure more than 80% of children with ALL in industrialized countries. Antileukemic medications, however, can cause significant adverse drug reactions. Moreover, some children have leukemia cell clones which are resistant to current antileukemic treatment [19].

The first studies focused on the effect of SNPs in genes encoding drug metabolizing enzymes in a treatment context. SNPs have identified great variability among individual responses to both efficacy and toxicity of different medications. While variability in the genetic efficacy and toxicity may be linked to clinical manifestations such as disease pathogenesis and severity, drug interaction, the emerging role of pharmacogenetic molecular diagnostic testing is increasingly recognized as pivotal in optimizing drug therapy [20].

Therefore, one of the best-established genotype–phenotype relationships is that of the *thiopurine* methyltransferase (TPMT) gene and its effect on thiopurine therapy for acute lymphoblastic leukemia and for immune modulation [21,22]. TPMT catalyzes the S-methylation, thus deactivation of thiopurines used in the treatment of ALL. Thereby, TPMT regulates the balance between cytotoxic thioguanine nucleotide and inactive metabolites in hematopoietic cells. Polymorphisms in the

TPMT gene have been extensively characterized [23]. TPMT-deficient patients are treated with 10- to 15-fold lower doses of these medications [24-27], they develop profound hematopoietic toxicity that precludes the administration of other chemotherapy and can be fatal [28]. Clinical interest in TPMT pharmacogenomics is based on studies showing that the TPMT genotype or phenotype can be used to identify patients at high risk of hematopoietic toxicity after thiopurine therapy [29].

So far the role of pharmacogenetics has been studied in analgesia [30,31]. A candidate gene is the hepatic cytochrome P450 gene CYP2D6, which catalyzes the metabolism of many drugs. One drug whose metabolism is strongly associated with CYP2D6 genotype or phenotype is the analgesic *codeine*, a prodrug that must be bioactivated to *morphine*, a strong opioid agonist, by CYP2D6. The efficacy and safety of codeine have been shown to be influenced by CYP2D6 polymorphisms (Table 2) [32,33]. A SNP affecting the action of analgesic agents is also SNP A118G in *OPRM1* (opioid receptor, mu 1) which causes a decrease in opioid potency by a factor of 2 to 3 [34]. The number of P450 genetic polymorphisms in relation to drug metabolism are illustrated in Table 2.

Table 2. Genetic polymorphisms and drug metabolism of cytochrome P450

P450 cytochrome	*Fraction of drug metabolism (%)*	*Frequency of genetic polymorphism*
CYP1A2	5	+
CYP2C9	10	+++
CYP2C19	5	+++
CYP2D6	20-30	+++
CYP2E1	2-4	+
CYP3A4	40-45	-

Drug efficacy is not influenced solely by variations in drug-metabolizing genes but also by polymorphisms in genes that encode drug receptors, transporters, and drug targets. For example, a common promoter variant in the molecular target of *warfarin* (VKORC1) strongly influences the dose levels required by individual patients. VKORC1 encodes the vitamin K-epoxide reductase protein, the target enzyme of *warfarin*. Variants in VKORC1 are significantly associated with warfarin sensitivity and reduced dose requirements (Table 3) [35]. Likewise, several transporters have been shown to have pharmacogenomic relationships with drug pharmacokinetics or effects.

For example, a synonymous SNP in the ABCB1 gene has been associated with the maximally achievable *digoxin* concentration [36].

Similarly, polymorphisms in the transporter SLCO1B1 have been associated with several phenotypes, including increased risk of *simvastatin*-induced myopathy [37], *methotrexate*-related gastrointestinal toxicity [38], and disposition of the cyclin-dependent kinase inhibitor *flavopiridol* [39].

Other studies have associated a CYP2C19 variant with diminished platelet response to *clopidogrel* [40] and CYP2C9 variant with warfarin dose requirements (Table 3) [41]. In acute leukemia, SNPs in the interleukin 15 gene are associated with disposition of antileukemic drugs [42].

Polymorphisms in cytochrome P450 gene CYP3A are associated to antiretroviral therapy [43]. CYP3A induction leads to an increased metabolism of the administered substance due to upregulated enzymes. This can cause adverse reactions, like inflammation of the liver (hepatitis) (Table 3) [44]. CYP3A is very polymorphic and metabolizes many of the drugs that are key components of highly active antiretroviral therapy

Table 3. Examples of personalized therapy approaches

Drug	*Gene*	*Clinical use*	*Phenotype*
Mercaptopurine	TPMT	Paediatric acute lymphoblastic leukemia	Myelosuppression
Tamoxifen	CYP2D6	Hormone receptor-positive breast cancer	Tamoxifen metabolism, progression-free and overall survival
Codeine	CYP2D6	Analgesia	Decrease in opioid potency
Warfarin	VKORC1, CYP2C9	Thrombosis, thromboembolism	Reduced dose requirements
Indinavir/ atazanavir	CYP3A	Viral infection	Hepatitis
Beta-lactam	TNF-α	Infections	Antibiotic allergy
Panitumumab/ cetuximab	KRAS, EGFR	Colorectal cancer	Worse prognosis
Gefitinib/ erlotinib	EGFR	Lung cancer	Response to therapy
Imatinib	CKIT	Gastrointestinal stromal tumor	Response to therapy
Trastuzumab	HER2	Breast cancer	Resistance to therapy

regimens. Pharmacogenomic investigations have examined the relationship between CYP3A4 and *indinavir/atazanavir*.

The variant G>A at -308 of TNFα correlates to IgE–mediated hypersensivity to beta–lactam. The TNFα GG genotype was a significant independent predictor of primary risk of beta–lactam allergy, concurrently with total IgE level. TNFα polymorphisms associate with risk of beta–lactam allergy (Table 3) [45].

Cancer-Therapy-Monoclonal antibodies

Cancer is a growing health problem around the world, particularly with the steady rise in life expectancy. More than 10 million cases of cancer per year are reported by the World Health Organization. According to the National Cancer Institute, an estimated 580,350 people died this year in the U.S.A. Cancer results from a multistage, multi-mechanism carcinogenetic process that involves mutagenic cell death and epigenetic mechanisms, during the three distinguishable but closely allied stages: initiation, promotion, and progression. Since reducing the initiation phase to a zero level is impossible, the most effective intervention would be at the promotion phase to eliminate premalignant cells before they become malignant [46-48].

Carcinogenesis is a complex process and both genetic and epigenetic factors are involved to cancer development. Epigenetic changes, such as DNA methylation, histone modifications and post transcriptional gene regulation by non-coding miRNAs are easily influenced by dietary and environmental factors [49]. These processes affect transcript stability, nucleosome positioning, and complete nuclear organization of the genetic material. Synergistically and cooperatively they determine whether a gene is silenced or expressed, as well as the timing and tissue-specificity of the expression of these genes [50].

DNA methylation is a well researched epigenetic mark that differs between normal cells and tumor cells in humans. In cancer cells, CpG islands preceding tumor suppressor gene promoters are often hypermethylated, while CpG methylation of oncogene promoter regions and parasitic repeat sequences is often decreased. However, in normal cells, CpG islands preceding gene promoters are generally unmethylated [51].

Cancer cells have been seen to exhibit decreased monoacetylated and trimethylated forms of histone H4 [52]. Loss of histone H4 Lysine 16 acetylation (H4K16ac), that is a mark of aging at the telomeres, specifically loses its acetylation and this histone acetylation loss might be battled with a histone deacetylase (HDAC) inhibitor specific for SIRT1, an HDAC specific for H4K16 [53].

In mammals, miRNAs regulate around 60% of the transcriptional activity of protein-encoding genes. Some miRNAs have also been found to undergo methylation-associated silencing in cancer cells [54,55].

Given the adverse effects and variable responses associated with cancer chemotherapy and the somatic genetic variation inherent in the biology of cancer, it is not surprising that some of the most promising applications of pharmacogenomics are found in oncology.

KRAS mutations are a predictor to resistance to pharmacologic inhibition of EGFR, such as the anti-EGFR monoclonal antibodies *panitumumab* and *cetuximab*. KRAS mutations are also associated with a worse prognosis (Table 3) [56,57]. Somatic mutations in EGFR are found to be present in most patients who respond to *gefitinib* and *erlotinib* treatment. It is postulated that these mutations, which cluster around the ATP-binding site of the tyrosine kinase domain (exons 18,19 and 21), stabilize the interaction between drug and the tyrosine kinase domain (Table 3) [58]. In lung cancer, the EGFR amplification, which is not as frequent as initially reported, is also associated with response to this treatment with cetuximab and gefitinib (Table 3) [59]. Patients with GIST (gastrointestinal stromal tumor), carrying *CKIT* mutations, respond to *imatinib*. Imatinib inhibits cell proliferation in tumors with mutated CKIT or PDGFR. However, CKIT activating mutation D816V is associated with *imatinib* resistance (Table 3) [60,61].

HER2 gene amplification causes gene overexpression and offers a response to therapy, in breast cancer and other types of cancer [62,63]. *Trastuzumab* was originally approved for use in HER2-positive metastatic breast cancer by the United States Food and Drug Administration (FDA) in 1998, based on a randomised phase III study, where the combination of *trastuzumab* and chemotherapy in previously untreated patients significantly improved the objective response rates (ORR), progression-free survival (PFS), and overall survival (OS) over chemotherapy alone (Table 3) [64]. Despite this notable success, 70% of patients with HER2-positive breast cancers demonstrate intrinsic or secondary resistance to *trastuzumab*, highlighting the importance of developing new therapies for this disease [65].

Discussion

Barriers between pharmacogenomic testing and clinical trials

As it was stated, the aim of pharmacogenomics is to elucidate functionally relevant genomic determinants for drug disposition and response in order to optimize drug therapy based on a patient's genomic profile. Pharmacogenomics has evolved from the study of single candidate genes to large-scale genome-wide strategies.

Proteomics has been also applied both in anticancer drug discovery and for personalized management of cancer. Examples of applications of oncoproteomics are given for cancers of various organs such as the brain, breast, colon and rectum, prostate, and leukemia [66].

The transition from pharmacogenomics to clinical practice seems necessary, and clinicians have a key role in this process. The use of genotype-guided therapies requires that clinicians will have a level of knowledge sufficient to understand and interpret the rationale for prescribing for certain genotypes, but not for others [67]. Clinicians have to know the influence of genetic variation in drug response and should be well educated on the clinical value, availability, and interpretation of pharmacogenomic tests. However, clinician acceptance is presently an unknown factor. It is possible that this may be a significant barrier to introduction of widespread genotype-based therapy. Recent surveys of pharmacists and physicians in the United States reveal that many feel inadequately educated in pharmacogenomics [68-70]. Results have also shown that more often clinicians who were well informed about the availability and potential applications of pharmacogenomic tests, incorporated pharmacogenetics into clinical practice [68]. Reported deficiencies included knowledge about the kinds of the tests available, how to procure them, and how to interpret and apply the results to a patient's care in the context of other clinical variables [68,71].

SNPs are very common in human genome, therefore millions of SNPs must be identified and analyzed to determine their involvement in drug response. Furthermore, our knowledge of which genes are involved with each drug response is limited. Since many genes are likely to influence responses, it is highly time-consuming and complicated to associate gene variations and drug response.

Another common problem is the limited drug alternatives. Since there are only one or two approved drugs available for treatment, patients carrying gene variations may be left without any alternatives for treatment. Also, the need for alternative drugs that serve only a small portion of the population and the great cost of bringing a new drug in market may be a disincentive for drug companies [72].

Cost is a potential barrier to the widespread implementation of pharmacogenetic testing into routine clinical practice. Test information will need to be balanced against other clinical (and cost) considerations, and a modified rather than strict gatekeeper model may be most appropriate [67]. While the costs of drug development to the pharmaceutical industry up to licensing will be reduced through a more efficient streamlined drug development process, the use of the drug after licensing will incur the combined cost of the drug and the pharmacogenetic test. Thus, cost-effectiveness of pharmacogenomic tests becomes an important parameter. In general, it is thought that the cost-effectiveness of health care technologies is primarily dependent on the cost and effectiveness of the technology, the morbidity and mortality associated with the phenotype, and the cost of treating the phenotype [14]. Several studies evaluated the clinical validity and utility of pharmacogenomic tests and provided support for the need to invest in implementation strategies [73-75]. A case study examined the cost–effectiveness of thiopurine methyltransferase (TPMT) genotyping prior treatment and showed that TPMT testing has a favorable cost–effectiveness ratio. This study indicated that TPMT genotyping should be seriously considered as an integral part of healthcare prior to the initiation of therapy with thiopurine drugs [74]. In addition, a recent study found that CYP2C9 and VKORC1 genotyping resulted in a 43% lower risk of hospitalization for bleeding or thromboembolism [76].

There is also the question of test location. Pharmacogenetic tests can be conducted in medical treatment (point of care testing, POCT) or by commercial laboratories. Current pharmacogenetic tests are conducted by specialist clinics. However, pharmacogenetic tests development for other clinical situations, such as general practice, may in practice be less acceptable. Technologies for genetic POCT will eventually become available, but this will require considerable investment in infrastructure and training.

A patient having a pharmacogenetic test may have implications in obtaining life insurance. Life insurance companies routinely use phenotypic

information to decide on insurance information. It is unknown whether pharmacogenetic information will not be used in a similar manner. It is likely therefore that in the future they will be given access to some information. For example, an individual who has a high risk of developing a disease, but has a favorable response genotype may actually have to pay lower premiums than an individual with a low risk of disease but with a genotype that indicates poor response to the drug.

Commercially available technology that is available for *in vitro* quantification of drug and drug metabolite levels in blood and plasma include high-performance liquid chromatography, mass spectrometers, flow cytometers, SPR biosensing instrument, ELISA.

However, these technologies are not practical for personalized medicine applications because they are expensive, require specialized reagents labeled with fluorescent or radioactive tags, semi-quantitative, time-consuming and unable to distinguish between substrates and inhibitors. Therefore, current screening of drugs and metabolism status of each patient on a daily basis is not yet practical. The solution to decentralization of hospital-based tests for the evaluation of drug metabolism is through design and development of new portable biosensor technology capable of evaluating pharmacokinetic parameters such absorption, distribution, metabolism, and excretion (ADME), along with measuring the toxicological effect of a drug in a real-time.

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