Pharmacogenetics in Clinical Practice

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Abstract: Pharmacogenetics is a discipline that investigates how genetic variation relates to the drug efficacy and safety. The goal of pharmacogenetics is a personalized treatment, where according to genotype we would be able to prescribe the most effective drug at the most appropriate dose for an individual patient. The aim of this review is to summarize pharmacogenetics as a specialization with its own background, research, methods, including barriers and promises for the future.

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The impact of pharmacogenetics on our ability to predict drug response is one of the most promising and fertile areas of genomic and personalized medicine (Ginsburg and Willard, 2009). Inter-individual variability in the response, intended or unanticipated, to similar doses of a given drug is an inherent characteristic of drug therapy. In everyday settings, it is clear that not all patients who are treated with the standard care have an equivalent pharmacological response. Some patients experience no treatment benefits, while in others the treatment is associated with undesirable side effects (Pickar and Rubinow, 2001; Kliegman, 2007). The interindividual variability contributes to the broad range of drug responses and the "optimal" drug that would be effective and safe for all patients does not exist (Riedlová and Richterová, 2008). The role of genetic factors in drug disposition and response is studied by pharmacogenetics/pharmacogenomics. In addition, environmental factors (e.g. dietary habits, smoking, co-medication, exposure to toxic substances), factors of physiological differences (age, sex, disease, pregnancy), and patient compliance contribute to variations in drug metabolism and responses. Therapeutic drug monitoring programs have been the earliest applications of personalized medicine; these programs recognize that all patients are unique and that the serum concentration-time data for an individual patient could be in theory used to optimize pharmacotherapy. Routine therapeutic drug monitoring, however, does not necessarily translate to improved patient outcome in all situations (Kliegman, 2007). Unlike traditional therapeutic drug monitoring (TDM), which is not performed until after a drug is administered, pharmacogenetics testing can be conducted even before treatment begins. Pharmacogenetic testing is universal for many drugs and it can be done just once. Based upon the evidence that disentangling environmental effects from genetic influence as sources of variability in drug response is not always possible, the combined use of classical TDM (as a phenotyping approach) and genotyping of drug metabolic capacity is currently considered to be the most sophisticated way to individualize the dosage of several drugs for the patient (Amos et al., 2007; Ginsburg and Willard, 2009; Gervasini et al., 2010; Crews et al., 2011).

Pharmacogenomic research

Over the past decade, pharmacogenetics incorporated into pharmacologic research and drug development initiatives in hope of improving medical care or economical benefits (Cohen, 2008; Sadee, 2011). The regulatory bodies throughout Europe as well as major regulatory agencies world-wide now widely recognize the importance of pharmacogenomic approaches during drug development. It is now evident that number of medicines that were granted marketing authorization status in recent years would not be licensed if there were no pharmacogenetic biomarkers for the prediction of efficacious and safe use. There are several scientific guidelines on pharmacogenomics issues drafted by the Pharmacogenomic

working party of the Committee for Human Medicinal Products at the European Medicines Agency, London (EMA). These guidelines define the terminology, and also discuss recommended ways on number of issues in pharmacogenomic methodology not only during pharmacokinetic testing, but also in the whole drug development program and postmarketing pharmacovigilance phase (EMA/CHMP/37646/2009, EMEA/CPMP/3070/01, EMEA/128517/06). These documents should be taken into the account in conjunction with other scientific guidelines of the EMA for multidisciplinary areas (e.g. determination of pharmacokinetics of new chemical entities) and specific guidelines for new drugs in defined treatment areas. In theory, the development of a new product may optimally involve prospective pharmacogenomic approach, i.e. defined by identification of pharmacogenomic biomarkers for drug efficacy are safety during phase I and II studies of the drug development with subsequent selective enrolment of subjects to phase III studies based on pharmacogenomic screening criteria. However, retrospective pharmacogenomic approach is far more frequent and although the retro-activity in drug development is in general a major limitation for the data, there may be no other option in defining and using pharmacogenomic biomarkers in drug development.

One possible cause of interindividual variability is genetic variation in pharmacokinetics. Of particular relevance are polymorphisms in genes encoding drug-metabolizing enzymes and drug transporters (Riedlová and Richterová, 2008). With respect to pharmacokinetics, the highest level of polymorphism is found in genes involved in drug metabolism; phase I metabolism of approximately 40% of clinically used drugs occurs via polymorphic enzymes (Phillips et al., 2001). Currently, the most important polymorphic enzymes are the cytochrome P450 (such as CYP2D6, CYP2C9, CYP2C19, CYP2C8, CYP3A4, CYP3A5), thiopurine methyltransferase (TPMT), uridine diphosphate glucuronosyltransferases (especially UGT1A1, UGT1A4, UGT2B7), N-acetyltransferase 2 (NAT2), dihydropyrimidine dehydrogenase (DPD) and organic cation transporter 1 (OCT1) (Ingelman-Sundberg, 2004; Slanar, 2005; Gervasini et al., 2010; Becker et al., 2011; Luxembourg et al., 2011). However, genes unrelated to the pharmacokinetic properties of the drugs are nowadays also known to affect the therapeutic performance or safety of the medicines. These genes may directly relate to the drug receptors/targets/transduction systems or may be only indirectly connected in pharmacodynamic mechanisms of action of the drug. E.g. the gene ADRB2 which encodes β2 adrenergic receptor and its two common non-synonymous SNPs (Arg16Gly and Glu27Gln) are intensively studied to clearly define their role in management of severe asthma (Chung et al., 2011; Tse et al., 2011).

Subpopulation prediction

We can divide patients into four classes according to their single locus genotype: "poor" or "slow metabolizers" (PM), "intermediate metabolizers" (IM), "extensive

metabolizers" (EM) and "ultra rapid metabolizers" (UM). Fast metabolizers are the most frequent in the population and they are considered as a standard group. Description of this classification including consequences of difference in gene pairs on the effectiveness/toxicity of drugs is shown in Table 1 (Ginsburg and Willard, 2009) and Figure 1 (www.icp.org.nz/icp_t8.html., 03/01/2012).

Table 1 – Pharmacogenetic classification

Class	Gene pair Graphical display of alleles	Anticipated impact on active drug	Anticipated impact on prodrug
Poor/slow metabolizer (PM)	Both members of gene pair contain variant that results in absent or non-functioning protein. ●●	Decreased efficiency in converting active drug to inactive metabolites. Increased risk for higher levels of active drug and clinical toxicity.	Inability to convert inactive prodrug to active metabolites. If the prodrug has no therapeutic properties, patient will then experience lack of efficacy despite drug dose increases.
Intermediate metabolizer (IM)	One member of gene pair contains variant that results in absent or non-functional protein and other member of gene pair contains variant that results in protein with reduced functions. Also, patient's pair of genes, each with variant that results in protein with reduced function or one member is resulting in protein with reduced function and the other member has sequence consistent with full functioning protein. ○●	Decreased efficiency in converting active drug to inactive metabolites. Increases risk for higher levels of active drug and clinical toxicity. However, if drug is normally started with a low dose and the dose slowly increased, effectiveness may be achieved sooner than in extensive metabolizers.	Decreased efficiency in converting inactive prodrug to active metabolites. Anticipate decreased effectiveness at standard maintenance dose.
Extensive metabolizer (EM)	Each member of pair has sequence consistent with full functioning protein. ●●	Active drug given at standard doses metabolized to inactive components, achieving effectiveness without or with minimal ADRs.	Prodrug converted to active metabolites achieves effectiveness without or with minimal ADRs.

Ultra rapid metabolizer (UM)	Locus inherited from one parent has gene sequence consistent with full function protein. Locus inherited from other parent has two or more copies of gene sequence resulting in full functioning protein or one member of gene pair has sequence consistent with full functioning protein and the other has variant that causes increased amounts of full functioning protein to be produced. ○○ ○○	Increased efficiency in converting active drug to inactive metabolites and higher risk for decreased effectiveness at standard doses.	Increased efficiency in converting prodrug to active metabolites and the associated increased risk for toxicity from higher than expected levels of active metabolites.

○ – wild type allele; ● – mutant allele

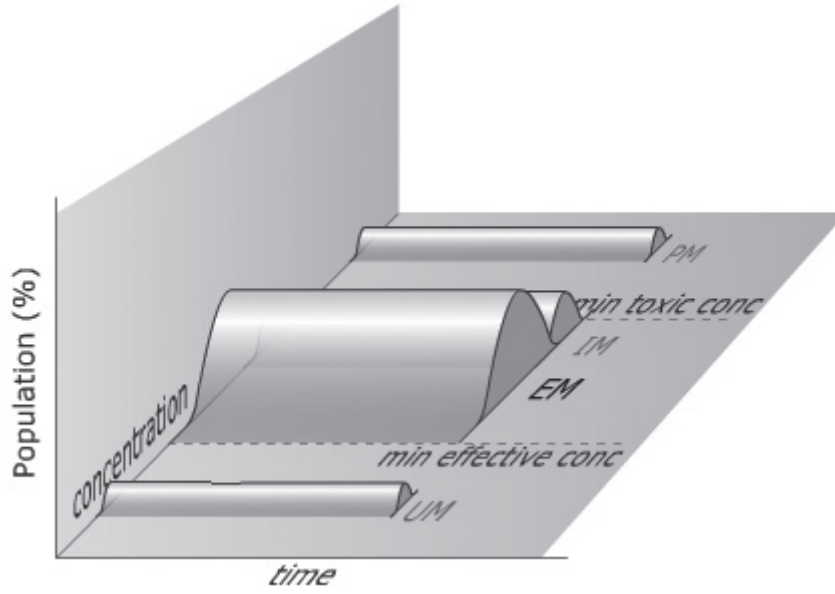

Figure 1 – *Pharmacogenetic classification (http://www.icp.org.nz/icp_t8.html).*

Methods of pharmacogenetic testing

The polymerase chain reaction (PCR), a procedure that exponentially amplifies a piece of DNA of specific size and sequence is the most used since its invention in 1985 by Karry Banks Mullise. Advantages of PCR are especially rapidity, specificity, sensitivity, sample accessibility, low cost and simplicity. In the simplest method, allele-specific PCR and the restriction of synthesized product by restriction enzymes are done followed by electrophoretic detection. More complex detection methods include fluorescence quantification, e.g. "real-time" PCR. The key feature of this method is that the amplified DNA is detected as the reaction progress in real time (Harris, 1998; Passarge, 2001). The limited capacity of PCR to SNP detection is solved by the introduction of microarrays. The number of SNPs which can be analyzed has been increased to hundreds of thousands of different sequences printed on the microarray. Other advantages of microarrays are automatization, rapid using, minimum of biological material and of course time saving. We can imagine microarray as a platform with thousands points (volume in the order of microlitres) containing probes of DNA. Every point represents a specific sequence of DNA which is characteristic for a specific allele. If the allele is present, it binds to the specific point with specific sequence of DNA and consequently fluorescent detection color binds to the point. This point starts to shine in ultraviolet light, so the presence of the specific allele can be determined. Results are evaluated by relative software (Karsten, 2006; National Research Council (US), 2007; Warner et al., 2011).

Clinical practice

The pharmacogenetic testing is provided for three main reasons. It is the selection of patients with the highest probability of therapeutic efficacy, reduction of adverse drug reactions (ADRs) and determination of the most appropriate drug dosage to provide efficacy and safety of the treatment. Recent evidence suggests that most prescribed medications are effective in no more than 60% of the individuals in whom they are used and a significant number of patients develop major adverse effects often leading to hospitalization. A wide range of pharmacogenetic tests have been recognized by the clinical and regulatory communities as having significant potential to alter standard medical practice. Information about genetic testing is now part of drug label for abacavir, warfarin, clopidogrel, irinotecan, maraviroc, cetuximab etc. (Riedlová and Richterová, 2008; Ginsburg and Willard, 2009). Table 2 contains a list of some clinically valid pharmacogenetic biomarkers and level of recommendation for related drugs in the context of FDA-approved drug labels (FDA – food and drug administration) and EMA recommendation (Gervasini et al., 2010).

Variability in pharmacokinetic characteristics

Irinotecan is a semi synthetic derivate of the natural alkaloid camptotecin and it is an important cytostatic drug in the treatment of colorectal carcinoma. Irinotecan is transformed to its active metabolite SN-38, a potent topoisomerase I inhibitor,

Table 2 – List of clinically valid pharmacogenetic biomarkers and level of recommendation for related drugs in the context of FDA-approved drug labels

Pharmacogenetic biomarker	Drug	Disease	FDA classification	EMA labels	The aim of genotyping
CCR5 expression	Maraviroc	HIV infection	+++	**	better efficiency
c-KIT expression	Imatinib	gastrointestinal stromal tumor	+	**	better efficiency
CYP2C9 variants, *VKORC1* variants	Warfarin	thromboembolism	++		elimination of ADRs
CYP2C19 variants	Vorikonazole	fungal infection	+		elimination of ADRs
CYP2D6 variants	Atomoxetine, fluoxetine	attention-deficit hyperactivity disease, depression etc.	+	*	elimination of ADRs
DPD deficiency	Capecitabin, 5-FU	colorectal cancer	+	**	elimination of ADRs
EGFR expression	Erlotinib	non-small-cell lung cancer	+	**	better efficiency
EGFR expression and *K-RAS* mutation	Cetuximab, Panitumumab	colorectal cancer	+++	**	better efficiency
G6PDH deficiency	Primaquine	malaria	+		elimination of ADRs
G6PDH deficiency	Rasburicase	hyperuricemia	++	**	elimination of ADRs
HER/NEU over expression	Trastuzumab	breast cancer	+++	**	better efficiency
*HLA-B*1502*a	Carbamazepime, phenytoin	epilepsy	++	*	elimination of ADRs
*HLA-B*5071*	Abakavir	HIV infection	++	**	elimination of ADRs
NAT variants	Isoniazid, rifampicin	tuberculosis	+	*	elimination of ADRs
Ph1 chromosome	Busulfan	chronic myelogenous leukemia	+		better efficiency
Ph1 chromosome	Dasatinib, imatinib	acute lymphoblastic leukemia	+++	**	better efficiency

Pharmacogenetic biomarker	Drug	Disease	FDA classification	EMA labels	The aim of genotyping
PML/RAR genes expression	Tretinoin	acute promyelocystic leukemia	+	*	better efficiency
TPMT variants	Azathioprine, 6-MP, thioguanin	acute lymphocytic leukemia	++	*	elimination of ADRs
UGT1 A1 variants	Nilotinib	chronic myelogenous leukemia	+		elimination of ADRs
UGT1 A1 variants	Irinotecan	colorectal cancer	++		elimination of ADRs

+++ – required; ++ – recommended; + – for information only; ** – included into indication or contraindication label information; * – included in the other label information; [a]for patients with an Asian ancestry

which is in turn conjugated by UDP (urine 5'-diphospho) glucuronosyltransferase 1A1 (UGT1A1) to a nontoxic SN-38 glucuronide. It was reported that the extent of this glucuronidation is inversely correlated with gastrointestinal toxicity and myelotoxicity, and suggested that the variability in this detoxification step is of genetic origin. A functional polymorphism was identified in the UGT1A1 gene (UGT1A1*28); this allelic variant consisted of an extra TA repeat (7 versus 6) in the promoter sequence and was associated with decreased glucuronidation rate. Carriers of the *28 are more susceptible to irinotecan-induced toxicity (Gervasini et al., 2010). Another example of pharmacogenetic testing is genotyping of gene for thiopurine S-methyltransferase (TPMT) before the treatment by thiopurines (e.g. immunosupressant azathioprine) because of the risk of potentially fatal hematological toxicity for poor metabolizers (Riedlová and Richterová, 2008; Slanar, 2008). Also genotyping of genes CYP2C9 and VKORC1 (vitamin K epoxide reductase) before the treatment by anticoagulant warfarin, that has an extraordinary low therapeutic index, is one of the most used in clinical practice (Riedlová and Richterová, 2008; Finkelman et al., 2011).

Variability in pharmacodynamic characteristics

An example of the drug where pharmacogenetic testing is a necessity for the effectiveness of the drug is maraviroc. Maraviroc is a CCR5 receptor antagonist class used in the treatment of HIV infection. It is also classified as an entry inhibitor. Maraviroc selectively binds to the human chemokine receptor CCR5. The rationale behind the use of CCR5-antagonists in treatment of HIV infection is based on the fact that HIV requires the binding to both the CD4-receptor and a co-receptor to enter a cell. The two relevant co-receptors are CCR5 and CXCR4. HIV can be either CCR5-tropic or CXCR4-tropic, so called tropism. Virus isolates replicating on both CCR5- and CXCR4-positive cells may do so either because they contain a mixture of R5- and X4-virus, or they use both CCR5 andCXCR4. If the CCR5

receptor is blocked by CCR5-antagonists, CCR5-tropic HIV cannot enter the cell. Due to its unique mode of action with exclusive activity against CCR5 tropic strains, viral tropism testing is mandatory before the drug is used in the clinic. At this case pharmacogenetic testing is not connected with patient, but directly with the virus, the cause of the disease (Vondrackova et al., 2011). However, the pharmacogenetic testing is often related to the character of the disease to determine inherent properties of the organism. This is the case for cetuximab, a monoclonal antibody having activity in the therapy of advanced colorectal carcinoma and in a variety of epithelial tumor types expressing the epidermal growth factor receptor (EGFR). Also trastuzumab, a monoclonal antibody that should be used only in patients with metastatic or early breast cancer whose tumors have either HER2 over expression or HER2 gene amplification as determined by an accurate and validated assay (Gervasini et al., 2010).

An extensive interindividual variability in drug response and voluntary use of opioid analgesic is well known for a long time. This interindividual variability is partly heritable, as reported in studies with twin pairs and in studies using models (Tan et al., 2009). Genetic factors can be based on more principles, e.g. pharmacokinetic characteristics of each opioid and potential polymorphism at metabolic enzymes. However the importance of polymorphism based on pharmacodynamics is almost the same for every strong opioid. The μ-opioid receptor, encoded by the OPRM1 gene, has been the subject of several genetic studies. OPRM1 is known to display several single nucleotide polymorphisms (SNPs). There is great interest in one common polymorphism of OPRM1, p. 118A/G, because the G118 allele has been shown to reset in the substitution of amino acid asparagine with aspartate at position 40 (Camorcia et al., 2012). Due to this discovery many clinical trials were conducted with following findings. The results showed that the 118G variant was associated with higher pain scores, higher morphine usage, and lower nausea score (Šerý and Didden, 2006). Other studies on pain sensitivity have also found that carriers of 118G are more sensitive to electrical stimuli and chemically induced pain, and also pressure pain. In terms of analgesic requirement, previous studies have shown that 118G carriers require higher amount of morphine to manage cancer pain, total knee arthroplasty, total hysterectomy, and major abdominal surgery. Another study on cancer patients also found that those carrying at least one copy of 118G were poorer responders to morphine and fentanyl (Tan et al., 2009). Higher doses of opioids are usually associated with worse safety profile of the drug.

Conclusion

The role of pharmacogenetics in personalized medicine continues to undergo profound changes, together with dramatic technological advances promising wider implementation in the future. Pharmacogenetics is constantly and rapidly developing. One of the possibilities how to receive the most recent information

about the new scientific findings and about phases of clinical development are electronic sources (Table 3). However, before the introduction of new discoveries into clinical practice, a big effort has to be employed. The issue in drug development is not only the identification of genetic biomarkers for drug efficacy and safety, but it also includes development and distribution of the methodology for reliable detection of these biomarkers in laboratories in real clinical settings, and also proper education of clinicians assuring the adherence to the pharmacogenetic screening if set in the indication limitations or recommendations. Despite the fact that pharmacogenetics is already starting to influence how physicians and scientists design clinical trials and despite its impact on the practice of medicine, the task of developing individualized medicines tailored to patient's genotypes poses still a major scientific challenge and offers many opportunities for research.

Table 3 – Internet resources containing pharmacogenetics information (11/07/2011)

Introduction into pharmacogenetics
http://www.ornl.gov/sci/techresources/Human_Genome/medicine/pharma.shtml
Risk haplotypes for a particular drug or a class of drugs
http://www.pharmgkb.org
Polymorphism of cytochrome P450
http://www.cypalleles.ki.se/
http://drnelson.uthsc.edu/CytochromeP450.html
Substrates of polymorphic enzymes
www.drug-interactions.com/
www.drugs.com/drug_interactions.php

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