

A NEW FARMACOGENOMICS APPROACH REGARDING THE VARIABILITY OF THE RESPONSE TO DRUGS IN DIFERENT HUMAN POPULATIONS

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Abstract: Pharmacogenomics is a new borderline medical discipline, which studies the interindividual variations of the enzymes, receptors and transporters involved in drug metabolism through the analysis of the involved genetic loci. In the present paper we expose some general considerations about the importance of this discipline in medical practice, emphasising the importance of human populations genome polymorphisms affecting drug efficiency and producing adverse reactions; eventually we expose the most recent trends in pharmacogenomics related to the subject.

INTRODUCTION

The differences noted in what concerns the efficacy of some therapeutically agents in normal humans and their risk of toxicity are determined by the presence of many allele which code for enzymes distinct in their metabolic activities. These constitutions are referred to as *genetic polymorphisms*.

Pharmacogenomics studies the impact of some inherited phenotypic traits in pharmacology and toxicology and analyses the loci involved in drugs metabolism with the purpose of identifying those alleles which are affecting drugs efficacy or determine adverse reactions. It is generally defined as a branch of pharmacogenetics, discipline with a broader content.

Pharmacogenomics is a high interest domain of study for both medicine and pharmaceutics; it generates, in the same time, hope and concern for physicians and reserchers involved in pharmaceutical industry. Pharmacogenomics is a reason of concern because many drugs already produced variable responses or even severe adverse reactions forcing the drug companies to withdraw their products from the market, but it also brings hoppe for an individualised treatment which will be described in the following chapters. Many pharmaceutical companies are trying to minimise their loses by testing their drugs metabolism using genetically variable enzymes.

Human genome polymorphisms and their effect on the response to drugs It is well known in the medical practice that some pharmacological agents are more effective fore some humans in comparison to other.

The individual variation of the response to drugs is a very important clinical problem; interindividual differences extend from the absence of the response to a specific pharmacological agent, till the sudden apparition of an adverse reaction.

The clinical consequences can vary from simple to severe symptoms, even exitus. A study performed in United Kingdom suggests that one in fifteen hospitalisations is due to adverse reactions to drugs; another study performed in USA estimates that 100,000 patients die and another 2.2 million are affected because of adverse reactions to drugs [11].

The differences noted in the response to drugs could be due to:

- variations in the rate of metabolism of a certain drug controlled by a given enzyme,
- the receptor molecule for the given drug has different affinities determined by genetic polymorphisms,

- a disease can be a mixture of different subtypes determined by distinct metabolic mechanisms.

The distribution of patients according to their response to drugs correlated with their receptors genotypes can be assigned to the following patterns:

- the group of patients refractory to the given treatment is more likely to contain mutant variants of drug receptors,
- the group of patients with a weak response to the given treatment is more likely to contain the normal variant of the receptor,
- the group of patients with an excellent response to the given treatment, in which are predominating the variants provided with a strong receptors hiperexpression.

Table 1: The frequency of the inefficient or refractory response to some classes of drugs (11)

Class of pharmacological agents	Inefficient or refractory response
Selective inhibitors of serotonin reuptake	10 – 25 %
Inhibitors ACE	10 – 30 %
Beta blockers	15 – 25 %
Tricyclic antidepressants	20 – 50 %
HMG Co reductase inhibitors	30 – 70 %
Beta 2 agonists	40 – 70 %

The variants of the gene involved in a given drug metabolism are responsive for the emergence of the following “populations” of individuals:

- “slow metabolisers” – bearers of a mutation that inactivates the gene responsive for drug metabolism,
- “normal metabolisers” – bearers of two copies of the normal gene,
- “rapid metabolisers” – bearers of many copies of the given gene (a gene amplification phenomenon which generates

Genetic variations in pharmacokinetic and pharmacodynamic effects in various human populations

Numerous factors, including genetics, affect drug metabolism and thus alter the bioavailability of therapeutic drugs. The best studied metabolizing enzymes are the cytochrome P450 (CYP450) isoenzymes, the N-acetyl transferase (NAT) isoenzymes, the UDP-glucuronosyl transferases, and the methyl transferases. Of these enzymes, the CYP450s are very important because they metabolize drugs into products that are readily excreted into the urine and faeces. In humans, six different forms of CYP450 (CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4) are largely responsible for eliminating drugs.

The rate of metabolism by several of the cytochrome CYP450 enzyme subfamilies varies, due to genetically-determined polymorphisms in all populations studied. Recent research using phenotyping and genotyping techniques has reflected the

interest and importance of these pharmacogenetic factors in determining drug responses. Some of the metabolizing enzymes such as CYP1A1, 1A2, 2A6, 2C9, 2C19, 2D6, 2E1, NAT1, NAT2 and NQO1 exhibit genetic polymorphism and alter responses to drugs (table 2).

These metabolic polymorphisms are determined by gender (e.g. CYP1A2) and racial/ethnic origin. Increased CYP1A activity (an enzyme catalysing a phase I oxidation reaction), coupled with slow acetylation (a phase II conjugation reaction), resulted in less myelosuppression from the active metabolites of the drug amonafide.

Because every individual represents a combination of drug-metabolizer phenotypes, given the large number of enzymes involved in drug metabolism, it is apparent that some individuals are likely to have unusual reactions to drugs, or to combination of drugs, due to the coincident occurrence of multiple genetic defects in drug-metabolizing enzymes. Such an alignment of genotypes, particularly when coupled with polymorphisms in drug receptors, is likely to constitute part of the mechanism for the so-called 'idiosyncratic' drug reactions.

Table 2: The most frequent pharmacogenetic polymorphisms of drug-metabolising enzymes in various human ethnic groups [6]:

Cytochrome P450 (drug oxidation): CYP2D6 Poor metaboliser White 6%, African American 2%, Oriental 1%
Codeine, nortryptiline, dextromethorphan. Ultra-rapid metaboliser Ethiopian 20%, Spanish 7%, Scandinavian 1.5% CYP2C9 reduced activity
Isoniazid, procainamide, sulphonamides, hydralazines Thiopurine methyltransferase (S-methylation) Poor metaboliser Low in all populations
Tolbutamide, diazepam, ibuprofen, warfarin CYP2C19 poor metaboliser Oriental 23%, White 4%
Mephenytoin, omeprazole, proguanil, citalopram N-Acetyl transferase (acetylation) poor metaboliser White 60%, African American 60%, Oriental 20%, Inuit 5%

Although no evidence to date suggests the CYP3A4 isoenzyme exhibits genetic polymorphism, in recent years there has been much discussion about the 3A4 system because of life-threatening arrhythmic side-effects that can occur as result of enzyme inhibition and accumulation of the antihistamines terfenadine, astemizole and cisapride. Terfenadine has been removed from the market because of its serious cardiovascular drug interactions.

Concerning CYP2C9, recent data suggest that patients who require low doses of warfarin (1.5 mg/day) carry point mutations (alleles CYP2C9*2 and CYP2C9*3) at the gene coding for CYP2C9 (which could occur at a frequency of 21% in the general population). These patients metabolized warfarin poorly, and responded to small doses of the drug with greater lengthening of the prothrombin time and higher international normalized ratio (INR) values than did carriers of the wild-type allele CYP2C9*1.

Genetically determined high-responders to warfarin had bleeding complications four times more commonly than did a control group stabilized on larger doses of the drug. Knowledge of carriage of the hyper-responsiveness alleles of CYP2C9*2 and CYP2C9*3 might help the clinician to decide against the use of warfarin (in favour of other coumarin derivatives such as phenprocoumon and acenocoumarol, the metabolism of which is less influenced by CYP2C9), particularly in high-risk elderly patients [7].

In addition to variation in drug metabolism or pharmacokinetics, the genetic variations in receptor function (and thereby pharmacodynamic effects) are important. Subtle differences in the sequences of receptor subtypes for dopamine, serotonin and catecholamines may result in individual differences in behavior and drug responses. Overall, a highly complex picture emerges in which genetic variation in both pharmacodynamic and pharmacokinetic factors contributes to drug responses. Some patients do not respond to a given drug because it is not processed efficiently; other patients do not respond because the disease gene defects or its pathway is not targeted by the drug.

Great progress has been made in understanding the molecular genetics of acetylation as well as the clinical consequences of being a rapid or slow acetylator. Inborn errors (several different alleles) at the hepatic arylamine N-acetyltransferase-2 (NAT2) locus are responsible for the traditional acetylator polymorphism. Rapid and slow acetylators reflect the genetically determined variation in the elimination of xenobiotics, as well as in NAT2 activity in the liver and other tissues. The human NAT2 gene contains an 870 bp intronless protein-coding region. To date, one allele with a code for fast acetylation (wild-type) and several mutated alleles with codes for impaired acetylation activity have been discovered. Of all the NAT2 allelic variants that had been identified, three (NAT*5, NAT*6 and NAT*7) account for majority of the slow NAT2 acetylator genotype in White subjects. N-acetylation status seems to be associated with several kinds of diseases, such as colon cancer, rheumatoid arthritis, and systemic lupus erythematosus [1].

The independent genetic feature as a rate of acetylation was shown to be related to the immunological system dysfunction. It may be one of the factors that makes an individual susceptible to the development of an atopic disease, and one study showed that up to 80% of individuals with chronic allergic rhinitis had a slow acetylation phenotype. A recent study which assessed the influence of NAT2 polymorphism on the risk of development of atopic disease also suggests that the risk of development of atopic diseases was five-fold greater for homozygous slow acetylators compared to healthy subjects, and that slow acetylation genotype may be an important factor of individual susceptibility to atopic diseases. This group of patients may also be at increased risk of adverse reactions after using drugs which are mainly metabolized by acetylation reaction. Among them, the mechanism of hypersensitivity to sulfonamides typical for slow acetylators seems to be of particular importance [4].

Consideration of the genetic characteristics leads to population segmentation into groups, the slow metabolizers (having a slow metabolism) and fast metabolizers (having a normal metabolism).

For example, in some Asian populations the incidence of poor metabolizers of the gastrointestinal drug omeprazole (due to polymorphism in CYP2C19) is 15–23%, compared to 2.5–6% in Caucasians. In individuals with a poor-metabolizer genotype for CYP2C19, the therapeutic efficacy of omeprazole (a proton-pump inhibitor widely used as acid inhibitory agent for the treatment of upper gastrointestinal diseases and metabolized by CYP2C19) may be increased. In patients with a poor-metabolizer phenotype or genotype of CYP2C19, the area under the plasma concentration-time curve of omeprazole is markedly increased, and the clinical effect of omeprazole is greater. Acid secretion in patients with a poor metabolizer status of CYP2C19 who are undergoing an omeprazole therapy is therefore assumed to be more strongly inhibited than those with the extensive metabolizer status. Cure rates for *Helicobacter pylori* were noted to be 28.6%, 60% and 100% in the rapid-, intermediate-, and poor-metabolizer groups, respectively [11].

The results of the genotyping test for CYP2C19 seem to predict the cure of *Helicobacter pylori* infection and peptic ulcer in patients who receive dual therapy with omeprazole and amoxicillin. A recent study designed to determine whether the effects of omeprazole on intragastric pH depends on CYP2C19 genotype status confirmed that after omeprazole administration, significant differences in mean intragastric pH values and plasma levels of gastrin, omeprazole and its metabolites were observed among the three groups of volunteers (homozygous extensive metabolizers, heterozygous extensive metabolizers and poor metabolizers), whereas no significant differences in these parameters were observed with the placebo administration. Both the individual omeprazole AUC and mean intragastric pH values were greater in the poor metabolizer group compared with those in the homozygous extensive metabolizer and heterozygous extensive metabolizer groups. The results confirmed that the effects of omeprazole on intragastric pH significantly depends on CYP2C19 genotype status, and also suggest that the genotyping test of CYP2C19 may be useful for an optimal prescription of omeprazole [6].

Low metabolic activity of the CYP2D6 enzymes is inherited as an autosomal recessive gene and although CYP2D6 represents only about 1.5% of the total liver enzymes, it is involved in the metabolism of a number of commonly used drugs. There are now more than 20 identified variant CYP2D6 alleles which contribute to the variation in CYP2D6 metabolism. The most common allelic variations associated with poor-metabolizers in Caucasians are CYP2D6*4 (75%), *3 (5%) and the gene deletion *5 (15%). For drugs in which CYP2D6 plays a predominant role in metabolism, poor-metabolizers will have high plasma concentrations and report the most severe adverse reactions [7].

Studies in Caucasian extensive-metabolizers and poor-metabolizers have uniformly demonstrated a 2- to 5-fold difference in the capacity to metabolize CYP2D6 substrates, such as antidepressants and neuroleptics. On the other hand, non-Westerners (Asians and Indians) may require lower doses of several classes of psychotropics that are metabolized by CYP2D6 (e.g. conventional neuroleptics and tricyclic antidepressants) than do Westerners. The poor-metabolizers lack this enzyme as a result of an autosomal recessively transmitted defect in its expression. When drugs are converted to an active

metabolite by 2D6 (e.g. conversion of codeine to morphine), the drug may be ineffective in poor-metabolizers. Although significant interactions between 2D6-metabolized drugs with the well-known inducers rifampin and antiepileptics have been described, specific inducers of 2D6 have yet to be clearly identified. Administration of dextromethorphan followed by measurement of O-demethylated metabolite excretion in urine is an accurate and non-invasive way of phenotyping individuals as either extensive-metabolizers or poor-metabolizers for 2D6 activity [6].

Many opioid analgesics are activated by CYP2D6, rendering the 2–10% of the population who are homozygous for non-functional CYP2D6 mutant alleles relatively resistant to opioid analgesic effects. It is thus not surprising that there is remarkable interindividual variability in the adequacy of pain relief when uniform doses of codeine are used [8].

Thiopurine methyltransferase (TPMT) is a cytosolic enzyme that catalyses the S-methylation of aromatic and heterocyclic sulfhydryl compounds, including the thiopurine drugs 6-mercaptopurine (6-MP) and 6-thioguanine. Thiopurines are used to treat patients with neoplasia and autoimmune disease as well as recipients of transplanted organs. The TPMT genetic polymorphism may represent a striking example of the potential clinical importance of pharmacogenetic variation in expression of a drug-metabolizing enzyme. Individuals with genetically very low levels of TPMT activity are at a greatly increased risk for potentially life-threatening toxicity when exposed to standard doses of thiopurines, while those with very high levels of this enzyme activity may be undertreated with the same dosages of these drugs [5].

Recent genetic data suggest that the active gene for the TPMT enzyme is ~34 kb in length, consists of 10 exons and has been localized to chromosome band 6p22.3. The wild-type allele for high TPMT activity has been designated TPMT*1, and to date eight variants for very low TPMT activity have been reported. [4] The most common of these in Caucasians, TPMT*3A, represents 55–70% of all variant alleles for very low activity. TPMT*3A contains two point mutations, G460A and A719G, resulting in Ala154Thr and Tyr240Cys amino acid substitutions, respectively. However, because of the clinical significance of inherited variation in levels of TPMT activity, characterization of as many variant alleles responsible for very low TPMT activity as possible will be necessary so that DNA-based diagnostic tests can be compared with the phenotypic test presently used to individualize therapy with thiopurine drugs. The ultimate aim is to minimize toxicity and improve the therapeutic efficacy of this important class of pharmacotherapeutic treatments [6].

Bronchodilator responsiveness to β_2 -adrenergic receptor agonists in patients with asthma varies considerably and several missense mutations in the coding region of the β_2 -adrenergic receptor gene have been identified. [1] Among the general population (including patients with asthma), β_2 -adrenergic receptor alleles are distributed in the following approximate proportions: homozygous Arg (Arg16/Arg16), 15%; heterozygous (Arg16/Gly16) 38%; homozygous Gly 16 (Gly 16/Gly 16), 45%; homozygous Gln27 (Gln27/Gln27), 26%; heterozygous (Gln27/Glu27), 49%; and homozygous Glu27 (Glu27/Glu27), 22%. The Gly6 allele has been associated with enhanced agonist-promoted β_2 -receptor down-regulation, whereas the Glu27 allele showed minimal down-

regulation compared with the Arg16 and Gln27 alleles. Although asthma is primarily an inflammatory disease of the airways, mutations in the β_2 -adrenergic receptor may be risk factors in certain asthma phenotypes [2].

The variation in cytochrome drug-metabolizing genes that correlates with patients' adverse response or non-response in clinical trials need to be considered. This information could be used to stratify clinical trials, leading to higher efficacy and limiting adverse reactions [3].

Ultimately, detailed information about each patient's genetic variants relevant to drug treatments might eliminate the use of ineffective or even dangerous treatments. Prognosis of patients will be more informed, because more precise information on the aetiology of the illness, its pathophysiology and the effectiveness of therapeutic interventions will be available. Thus, the incorporation of pharmacogenetic information into trials as early as possible is recommended and appears very useful for effective drug development [9].

CONCLUSIONS

New gene targets for therapeutic intervention only provide a starting point in the long and difficult process of drug discovery. However, genomics will have an important impact in the later stages of drug development, especially in providing an understanding of the molecular nature of diseases and of the responses, both desirable and adverse to drugs. Modern genetics will bring about significant improvements in the provision and practice of healthcare by redefining disease and targeting treatment. It will also lead to the discovery of novel targets and effective treatments and the provision of more effective preventative healthcare [10].

Pharmacogenomics has major implications both for drug development and clinical management. Pharmacogenomics aims to complement the current 'one-medicine-fits-all' scenario with drugs that are based on a deeper understanding of gene variations and the effect of such variations on drug responses [9]. Drugs that are more specific, not only in terms of the particular molecule they target, but also in the populations they affect, will be much more widely accepted and used in the future. In the short term, pharmacogenomics will be strategically used for clinical development of particular compounds with potential efficacy or toxicity issues.

The therapeutic industry will soon be entering a time when solutions to therapeutic problems can be targeted to the individual. Using knowledge of gene functions and commercially available genomics tools, a genomics consumer will be able to employ focused, high-speed technologies that will produce an individualized treatment in a short period of time. This is a fundamental change in research and clinical medicine.

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