ECOGENETICS AND PHARMACOGENETICS: THE IMPORTANCE OF GENETIC POLYMORPHISMS IN THE VARIABILITY OF ORGANISMS RESPONSE TO ENVIRONMENTAL FACTORS

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Abstract: Genetics and genomics are certain to have a large impact in drug development and proper pharmaceutical treatment of subgroups of patients with many specific diseases. We should be able to increase the therapeutic margin for many agents. Genetic variation will also be important in refining estimates of risk from all kinds of environmental agents and in choosing more effective and more cost-effective risk reduction strategies. The linkage of information about genetic variation and information about environmental, nutritional, behavioral, metabolic, medical, and healthcare factors will be necessary to interpret the variation in clinical and public health terms. However, there is a great risk that present efforts to protect confidentiality and privacy of individual genetic information may make such research infeasible. In the present paper we expose some general considerations about the importance of the borderline disciplines which are studying the cited aspects (ecogenetics, pharmacogenetics and pharmacogenomics), 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

George Brewer of the University of Michigan introduced the term "eco-genetics" in 1971 , and many others helped develop this field (Motulsky G., 2002). As described in many papers, there are striking examples of genetic variation in responses to foods, food additives, alcohol, cigarette smoking, and other agents, as well as pharmaceuticals. Various state regulatory agencies are now increasingly interested in research data about variation in susceptibility within highly heterogeneous human populations, to improve the basis for health protection and to replace arbitrary, generally extremely conservative safety factors and related assumptions in risk estimates (Omenn A.G., 2001). This approach aims to overcome the predominant regulatory strategy of dealing with one chemical at a time, in one environmental medium (air, water, food, soil), and each health risk (cancer, birth defects, liver toxicity) in isolation. During the past decade there were issued numerous recommendations for each of the various agencies that regulate chemical hazards and reinforced strategies for risk communication.

One of the major challenges arising from studies of polymorphic genetic variation with particular cancers or other diseases is interpreting the inconsistency of associations reported; such as an experience with multiple ancillary studies in the lung cancer chemoprevention trial, CARET (Omenn G.A., 2001). Partly this problem reflects ethnic differences in gene frequencies and marked heterogeneity of the causes of common diseases. However, some combinations of P450 and glutathione S-transferase variants, or combinations of N-acetyltransferases and smoking history, shed light on what will surely become a general phenomenon: that we must investigate an array of relevant genes and the interacting environmental factors, not just single genes in isolation, to understand the predispositions to common diseases. The new technologies presented surely will facilitate such research and permit its application in medicine, public health, and environmental policy as we moved into the new millennium.

Even it is a branch of ecogenetics, pharmacogenetics is older and much “richer”; the origin and development of pharmacogenetics are traced with emphasis on early hints by Garrod, Haldane, and later by RJ Williams. The field was delineated by Motulsky in 1957 and described as pharmacogenetics by Vogel in 1959. Kalow's monograph (1962) definitely established the discipline. Resemblance of identical twins in drug metabolism as compared with non identical twins (Vesell, 1970) established the general importance of polygenic inheritance in disposal of many drugs. Ecogenetics was defined by Brewer in 1971 as dealing with genetic variation affecting the response to any environmental agents with emphasis on xenobiotics. More recent developments have broadened pharmacogenetic approaches to include novel genomic techniques with introduction of the term pharmacogenomics in the 1990's (Motulsky, 2002).

Genetic and genomic approaches (toxicogenetics and toxicogenomics) are also being applied in the "environmental genome project". The interaction of genetic variation with dietary factors led to the field of Nutritional ecogenetics (Nutrigenomics) which relates the role of genetics to nutritional requirements and nutrition-mediated susceptibility to chronic disease. The total promise of pharmacogenomics is often overstated. The field is likely to have an impact on choice of drug therapy and avoidance of adverse events but is unlikely to lead to a revolution in therapeutics. Aspects of pharmacogenomic approaches and its applications including problems of premature commercialization are discussed.

ECOGENETICS: THE ROLE OF GENETICS IN ENVIRONMENTAL RISK ASSESSMENT AND RISK MANAGEMENT

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.

Ecogenetics is dealing with the role of such genetic polymorphisms in the variability of organisms response to environmental factors. The result of such variability is a genetic vulnerability (susceptibility) in front of the environmental agression. Ecogenetical diseases are produced by the interacion of genetic vulnerability and environment agression.

In accordance with the environmental agents involved, one may describe the following domains:

- infections ecogenetics
- nutritional ecogenetics
- physical ecogenets
- chemical ecogenetics (including the most developped branch pharmacogenetics.

Knowledge from pharmacology and toxicology can be linked on a mechanistic basis to anticipate the polymorphic biotransformation enzymes and polymorphic receptors and other sites of action that would be relevant to new drugs and to environmentally encountered chemicals. An example is the metabolism of benzo(a)pyrene, a pro-carcinogen of the polycyclic aromatic hydrocarbon class of compounds common in combustion effluents and cigarette smoke. Benzo(a)pyrene is activated successively by cytochrome P450s and mitochondrial epoxide hydrolase to the benzo(a)pyrene-9, 10-diol-epoxide, the potent carcinogenic intermediate; several pathways serve to detoxify the carcinogenic derivatives (Omenn G.A., 2001).

Variation in susceptibility to chemical, infectious, and physical agents encountered in the workplace and in other environments increasingly is being recognized as an important variable in environmental and occupational medicine and environmental risk management.

PHARMACOGENETICS AND PHARMACOGENOMICS: 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 (Wolf et al., 2000).

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.

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.

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 phenoprocoumon and acenocoumarol, the metabolism of which is less influenced by CYP2C9), particularly in high-risk elderly patients (Meyer U.A., 2000).

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 (Tudose and Patraş, 2003).

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 (Farlow C.A., 1996).

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 (Kallow W., 1997).

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 (Wolf et al., 2000).

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 (Levy A.R., 1993).

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 (Meyer U.A., 2000).

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 (Levy A.R., 1993).

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 (Mungiu et al., 2000).

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 (Katz D.A.., 2002).

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 (Kallow W., 1997) 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 (Levy A.R., 1993).

Bronchodilator responsiveness to $ß_2$-adrenergic receptor agonists in patients with asthma varies considerably and several missense mutations in the coding region of the $ß_2$-adrenergic receptor gene have been identified. Farlow C.A., 1996 Among the general population (including patients with asthma), $ß_2$-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 $ß_2$-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 $ß_2$-adrenergic receptor may be risk factors in certain asthma phenotypes.

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 (Kuivenhoven A.S., 1998).

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 (Ruano-Ravina et al., 2002).

ETHICAL AND LEGAL IMPLICATIONS

Much of the excitement surrounding pharmacogenomics stems from the possibility of improving the safety and efficacy of drug interventions. Additionally, by conducting clinical studies in genetically homogeneous populations, it should be possible to use smaller, faster, and cheaper clinical trials. There is even the possibility that certain drugs that have failed clinical trials in broad populations could be "rescued" and demonstrated to be safe and effective when used only by individuals with certain genotypes.

Along with the mixture of hype and hope, the reality is that pharmacogenomics presents a number of challenges from an ethical, legal, and policy standpoint. Among these challenges are 1) the ethical, economic, and policy implications of market segmentation, 2) the ethical and social issues surrounding research in pharmacogenomics, including the generation of sensitive genetic information, and 3) the political challenge of ensuring equal access to beneficial pharmaceutical products developed through pharmacogenomics .

Most of the pharmacogenomic research is currently at the preclinical stage. Both at this stage and the later clinical research stage, an important, but generally unexplored, issue is whether the target population is supportive of the research. In particular, it is important to consider 1) whether individuals are willing to participate in research by donating biological samples and sharing medical records with investigators, 2) whether individuals are willing to undergo genetic testing as part of the research process, 3) whether individuals have suspicions about the medical research establishment, 4) whether concerns about privacy and confidentiality will cause individuals to decline to participate in research, and 5) whether individuals are concerned about the morality of research into human genetic variation. Although these concerns are certainly common to all

clinical trials, they warrant mention here because of the unique concerns centering around genetics in general (Omenn G.A., 2001).

As the research proceeds to the clinical stage, it will be important to develop inclusion and exclusion criteria based on genotype. One important issue is the ethics of including random or "nonmatched" controls in the studies (i.e., individuals whose genotypes do not suggest favorable responses). Informed consent will also be a major concern, including how researchers inform potential participants about the possible economic and social consequences of the research, including possible group-based harms. In this regard, the idea of community consultation before performing research in discrete ethnic groups has been debated in the literature.

From a policy perspective, as pharmaceutical companies segment the market, it may become economically impossible to pursue drug development for individuals with rare genotypes. Consequently, some governmental subsidies akin to those under the Orphan Drug Act may be necessary to encourage the development of "small market" drugs (Omenn G.A., 2001).

Over the next several years, as pharmacogenomic based medications become available, will managed care plans include them in their formularies? Most companies can be expected to undertake a detailed cost-benefit analysis to determine whether the incremental benefits are worth the incremental costs. Even if they are cost-effective, it remains to be seen whether the costs will be borne by consumers or third-party payers and how increased pharmaceutical costs will affect access to health care in general.

Finally, whenever the standard of care in medicine changes there is an increased possibility of liability for those providers who fail to meet the new standard of care. For physicians, the range of possible liability issues includes the failure to order the appropriate genetic tests or to interpret them and explain them to patients properly, the duty to warn patients of possible genotype-specific side effects of medications, and the possible issue of failure to warn at-risk relatives. To meet this heightened standard of care it will be necessary to include instruction in pharmacogenomics in schools of medicine, nursing, pharmacy, and other health care fields, as well as to include new developments in continuing education courses. Pharmacogenomics is a very promising avenue of research, but we must be careful to make sure that there are no unintended social consequences from introducing this technology (Omenn G.A., 2001).

CONCLUSIONS

Building upon the historical approaches taken in the field of eco- and pharmacogenetics, the information resource gained from the completion of the Human Genome Project, coupled with the development of high-throughput technologies, the development of sophisticated analytical tools, and the identification of relatively specific in vivo phenotypic markers, provides the potential for this field to make rapid advances.

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.

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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