THE ROLE OF PHARMACIST IN DEVELOPMENT OF PHARMACOGENETIC TESTING AND IMPORTANT UPDATES OF PHARMACOGENOMICS

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Summary

The field of 'pharmacogenetics', which is 'the study of variability in drug response due to heredity', should help in reducing drug-caused morbidity and mortality. The recently coined term 'pharmacogenomics' usually refers to 'the field of new drug development based on our rapidly increasing knowledge of all genes in the human genome'. However, the two terms – pharmacogenetics and pharmacogenomics – are often used interchangeably. The pharmacist, as well as the medical genetics counselor, should be knowledgeable in the rapidly expanding fields of pharmacogenetics and pharmacogenomics. The history of Pharmacogenetics, Drug polymorphisms, phenotype-genotype and its Correlation, Single Nucleotide polymorphism (SNP) and the role of Pharmacist in development of pharmacogenetic testing are discussed in detail. Our current appreciation of the degree of variability (including single-nucleotide polymorphisms, SNPs) in the human genome is described; ethical considerations and the possibility of a total genome scan at high resolution termed as Quantitative trait loci (QTL) mapping are also highlighted in this article. Finally, Drug metabolizing enzymes (DMEs) and individual's risk of cancer are also discussed in detail. Thus the need for pharmacist to keep themselves abreast of the latest information in the fields of Pharmacogenetics and pharmacogenomics is emphasized.

Key words: Pharmacogenomics, Pharmacogenetics, Single Nucleotide Polymorphism, Pharmacist

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Introduction

The term 'Pharmacogenetics' was first used in the 1950's to describe clinical observations of inherited differences in drug effects. It now describes the study of how inter individual variations in DNA sequence are related to drug response [(1)]. The use of genetic markers in healthcare is not a new phenomenon, but has been used for years e.g., in organ transplant and blood transfusions. The completion of Human genome project (HGP) is one of the greatest scientific achievements of the past 50 years. This project, which was completed in 2003, identified the thousands of protein-coding genes in the human genome and sequenced billions of chemical base pairs that make up human DNA. Genetic make up is broadly similar in humans, regardless of gender or ethnicity. However there are small variations in the genetic code, referred to as single nucleotide polymorphism (SNPs), which can have a profound effect on how an individual develops disease or responds to a medicine [(1-4)]. The HGP identified over 1.4 million SNPs with at least 60,000 of them in the coding region of genes [(4)]. Research in Pharmacogenetics has gained momentum in recent years, fuelled by these findings. It is hoped that increased knowledge in this field will allow genetic information to be used to inform prescribing decisions and allow more accurate prediction of drug safety and efficacy in individual patients. However, despite it being over 50 years since the conception of Pharmacogenetics, most clinicians still prescribe on a 'one drug fits all' basis. The potential in this filed is yet to be realized, but there are several current examples of how pharmacogenetic testing is improving patient care [(1)]. The application of Pharmacogenetics broadly falls into using genetic information to test for variation in an individual's germline DNA (the inherited genetic make-up of every cell in the body), which may, for example, determine the activity of a drug metabolizing enzyme and analyzing the DNA of tumour cells (this may be different from cells in the rest of the body and not inherited) [(1)]. In the foreseeable future, pharmacogenetic research will lead to the development of drugs that are more cost-effective, and medicines that respond better to patients' needs [(5)]. Several stakeholders positively support Pharmacogenetics and are attempting to drive forward scientific understanding and technological development in this area. These include the pharmaceutical industry, which is presented with the challenge of bringing new drugs into an increasingly competitive market [(5)]. In addition, the government faces mounting costs for delivering healthcare, so continues to look for solutions that improve the cost effectiveness of pharmaceuticals. Patient group may also show an interest in Pharmacogenetics developments if they offer improvement to the medicines selection process and result in improved drug efficacy and reduced ADRs. However the scientific community has not been universally enthusiastic about the potential of Pharmacogenetics. Experts have urged caution in light of unsubstantiated claims and calls have been made for a greater level of realism regarding the expectations of pharmacogenetic applications. This is primarily because, despite the significant progress in the mapping of the human genome, the connection between genotype and phenotype in drug response is complex. It is rare that one gene is responsible for a patient's response to a medicine. Usually, the inherited response is determined by the interplay of several genes, all of which encode proteins involved in multiple pathways of a drug's metabolism, disposition and effect. To understand the potential of Pharmacogenetics one need to know the basics of Phenotype, genotype and polymorphism in detail, which we will emphasize more & discuss in this section.

History of Pharmacogenetics

Some have suggested that Pharmacogenetics originated during this decade as a result of the Human Genome Project. Others suggest that Pythagoras – in Croton, southern Italy, about 510 B.C. – was the first to recognize the 'dangers of some, but not other, individuals who eat the fava bean', the adverse reaction being hemolytic anemia in those who are deficient in glucose-6-phosphate dehydrogenase. Still others [6, 7] propose that Snyder's original study in 1932, concerning the 'phenylthiourea' phenotype inherited as an autosomal recessive trait [8], represents the dawn of modern pharmacogenetics. Friedrich Vogel [9] first coined the term pharmacogenetics in 1959. Today, pharmacogenetics and pharmacogenomics represent studies on the fundamental gene–drug relationships ranging from genetic and genomic information, through molecular and biochemical mechanisms, and through a functional physiological and pharmacological context, in association with clinical studies. 'Pharmacogenetics' is 'the study of variability in drug response due to heredity'. In the past several years the term 'pharmacogenomics' has been introduced; this field emphasizes the development of novel drugs based on newly discovered genes as the entire human genome becomes sequenced. The two terms, however, have also been used interchangeably. The study of pharmacogenetics and Pharmacogenomics presents opportunities to colleagues working at a number of research levels, ranging from the most molecular to the most clinical.

These research fields include pharmacology, toxicology, molecular biology and human genetics, genomics, internal medicine, endocrinology, physiology, epidemiology, statistics, bioinformatics, and computational biology. At medical centers around the world, numerous ongoing clinical trials are being carried out in which the clinician gives a certain drug to patients in order to treat a particular disease; the clinician frequently observes that the drug either is not efficacious or is actually toxic to a certain subset of the patients, and the clinician has little expertise in pharmacology or human genetics.

It would be beneficial, therefore, to bring investigators like trained pharmacists, with backgrounds in pharmacology, molecular biology, and genetics, together with these clinicians in a research framework, so that functional variation in gene products (enzymes, proteins) that play essential roles in determining variability in drug responses can be studied, interpreted, and related to clinical research situations in a rapid and efficient manner. The ultimate outcome would be to reduce drug-caused morbidity and mortality worldwide.

Drug polymorphisms

Genetic polymorphisms exist in a human population when allelic variants occur with a frequency of 1% or greater. When drugs enter the body, their fate is affected by uptake, binding and distribution, biotransformation (metabolism), and excretion. The majority of pharmacogenetic differences that have so far been characterized on a molecular basis represent variability in Drug metabolizing enzymes (DME) metabolism; most of the remaining appears to represent alterations in receptor affinity, transporters, or protein binding [10-12]. Pharmacogenetic differences in uptake or excretion of drugs are relatively uncommon. Vitamin B12 malabsorption or hemochromatosis, and certain aminoacidurias (e.g., cystinuria) might be regarded as pharmacogenetic differences in uptake and excretion, respectively [6, 13, 14].

Phase I and phase II metabolism

Virtually all therapeutic agents are metabolized by 'phase I' (functionalization, often cytochromes P450), followed by 'phase II' (conjugation) DMEs. The human genome is expected to contain at least several hundred DME genes; for example, 49 human cytochrome P450 (*CYP*) genes are known to exist [6, 15]. Most incoming drugs might be regarded as 'exogenous signals' that are 'detected' by the cell – either by means of well-characterized endogenous receptors or by 'reception mechanisms' not yet understood; these drugs/signals can displace the naturally occurring endogenous ligands and act either as agonists or antagonists to up- or down-regulate phase I and phase II DME genes [16]. For example, carbamazepine and phenytoin are both known to up-regulate their own phase I metabolism; phenobarbital induces particular genes of both the phase I and phase II categories.

The oxygenated reactive intermediates following phase I metabolism, as well as many incoming nonmetabolized drugs (and even conjugated products whose conjugation group has been cleaved), are capable of causing toxicity. Toxicity is now appreciated to occur basically via two mechanisms: (a) oxidative stress, leading to perturbation of the cell cycle, and (b) covalent binding to cellular proteins and nucleic acids. Beyond the scope of this review, oxidative stress, disturbance of the cell cycle, and covalent binding can also lead to mutations and cancer. The drugs are transported into the cell, and the nonmetabolized drugs, reactive intermediates, and conjugated products can all be transported out of the cell. Genes encoding phase I and phase II DMEs, and the DME receptors, are known to exhibit polymorphisms, and thus are the basis of genetic variation in drug response [17–26]. Genes involved in the oxidative stress response and cell cycle regulation also might be expected to show polymorphisms, but these are less well understood or appreciated at the present time.

A common misconception, stemming from the way pharmacology and toxicology have been taught during the past several decades, is that DMEs, DME receptors, and drug transporters exist largely – if not completely – in the liver; this could not be further from the truth [6,15]. CYP3A4, the most abundant hepatic cytochrome P450, is also present in large concentrations in the gastrointestinal tract [6, 15]. Many DMEs exist in the vascular endothelial cells and contribute to the arachidonic acid cascade, cell division, inflammatory response, vasoconstriction, and numerous other homeostatic mechanisms [16]. DMEs exist in the brain and play roles in neuroendocrine functions. Particular CYP activities have been found to be as much as 50-fold greater in the human oral mucosa than in the liver [27]. A number of DMEs at high concentrations exist in the nasal mucosa [28]. Pharmacists must therefore keep in mind that variability in drug response (efficacy, toxicity) can occur in any tissue and need not be determined by hepatic DMEs, DME receptors, or drug transporters.

Phenotype and Genotype

The objective of any person working in the fields of pharmacogenetics and Pharmacogenomics is to consider in each patient how to relate a drug response (phenotype) to the genotype (Table 1). How can these correlations be made unequivocally?

First, it is necessary to define a quantifiable clinical drug response. Second, one needs to detect a nucleotide change (polymorphism). Last, one must prove, functionally or with power statistics, that this particular genotype is indeed responsible for the phenotype being studied.

In classical genetics, the 'phenotype' is usually defined as a visible trait – such as red hair or blue eyes. It is possible, however, to define phenotype in any quantitative clinical terms that you wish; for example, the 'sensitive phenotype' might be 'liver toxicity (or certain level of a particular hepatic enzyme denoting toxicity) after 1 month of drug ''X'' given at ≤ 2 g/ day, and the 'resistant phenotype' might be 'no evidence of hepatotoxicity after 3 months of drug ''X'' given at ≥ 3 g/day. As another example, the 'sensitive phenotype' might be defined as 'lung cancer before age 50 years combined with ≤ 60 cigarette-pack years of smoking history', whereas the 'resistant phenotype' might be defined as 'no evidence of malignancy in patients over the age of 75 years and ≥ 100 cigarette-pack years'. In other words, our definition of 'phenotype' is entirely arbitrary and it is best that the one group ('sensitive' or 'low') be unequivocally separated from the other group ('resistant' or 'high'), as will be discussed in more detail below.

It must also be emphasized that all the above mentioned traits, indeed virtually all human diseases, represent multiplex phenotypes (i.e., are polygenic, or derived from the contribution of two or more genes). What is the best experimental way to dissect these polygenic traits? If an entire population is examined, say, for toxicity as a function of drug dosage, there will be a general increase in toxicity – with means and standard deviations of the means for each level of drug dose. If outliers can be found, however, these extreme genetic variants are far more useful to the investigator who seeks to identify a mechanism for the trait than examining every patient in the general population.

The 'resistant outlier' represents an individual with little or no toxic response to a relatively large dose of drug, whereas the 'sensitive outlier' exhibits an exaggerated toxic response to a low dose of drug.

Studies involving a large dose range are normally not ethically possible in human clinical trials. Therefore, it is proposed that pharmacists might quantitate the pharmacogenetic phenotype in much the same way as genetic studies have been done in the dissection and identification of genes responsible for blood pressure homeostasis [(29–37)].

Based on the above discussion, it should be obvious that, if a patient has not been treated with a particular drug, we would not know his/her phenotype with regard to sensitivity or resistance toward this drug. This is a common corollary in Pharmacogenetics [(17–26)]. Although a patient might have the underlying genetic predisposition to be particularly 'sensitive' or 'resistant' to any drug or other environmental agent, unless the patient has been sufficiently challenged with this chemical (or, in the near future, genotyped by DNA testing), we might never know his/her covert genotype with regard to a specific metabolic (or receptor or transporter) pathway.

Single Nucleotide polymorphism

Occasionally one of the nucleotide in a DNA sequence may change. This is known as a single nucleotide polymorphism (SNP). If the SNP occurs in the coding region, this can lead to an alteration in the aminoacid sequence of the encoded protein and, potentially, a protein with altered function. This can affect Pharmacodynamic or pharmacokinetic processes.

It now appears possible to classify SNPs into three groups. (a) Coding-region SNPs (cSNPs), i.e., those that alter the amino-acid sequence of the encoded protein, are most likely to influence disease. Given an estimated ≈75000 genes in the human genome and about four cSNPs on average/gene, there are an estimated 240000–400000 common cSNPs in the human genome, and the typical person might be heterozygous for about 24000–40000 aminoacid altering mutations [(37)]. (b) Perigenic SNPs (pSNPS; i.e., inside or in the immediate vicinity of genes) include silent codon mutations and changes in the noncoding regions of the mRNA, all introns, the 5% flanking sequence – from the 5%-most enhancer (shown to be functional) to the transcription initiation site – and at least 100 bp 3%-ward of the last exon. Noncoding DNA adjacent to coding regions appears to be functionally constrained to a significant degree, perhaps comparable to that in the coding region, suggesting another 250000– 500000 pSNPs in the human genome [(37-40)]. (c) Random noncoding SNPs (rSNPs) occur in intergenic genomic ('junk') DNA, are the result of random 4-fold degenerate sites, and will in all likelihood make up the remaining 5–29 million SNPs in the human genome.

It is experimentally possible to prove that a particular cSNP is unequivocally correlated to a pharmacogenetic phenotype. At the present time it is possible – but difficult – to prove, by power calculations [(40)], that particular pSNPs are associated clinically with variation in drug response; with alleles having frequencies of < 5%, however, it should be emphasized that demonstration of such associations will typically require thousands of patients. These types of studies will perhaps be possible only through collaborative efforts with large pharmacogenetic centers worldwide.

Many SNPs are being found to segregate together (i.e., in strict linkage disequilibrium). For example, a SNP 3673 bp upstream from the 5′ end of the gene, a SNP in exon 3, and a SNP in intron 19 might be found always to go hand-in-hand [e.g., the 13 identifiable distinct haplotypes representing unique SNP patterns in the Rieder et al. [(36)] study]. Such data are already being used in the field of criminology, as well as anthropological studies, to estimate tribal migrations and divergence of ethnic groups. By the rapid-throughput sequencing and re-sequencing of innumerable genes (e.g., using the latest DNA sequencer models or DNA chip technology), such data will become abundantly available during the next several years. A recent study of the human *CYP*2*D*6 gene [(41, 42)], which described '48 mutations and 53 alleles' in a screen of 672 unrelated individuals, further underscores the urgency of the need for some consensus soon on (a) how to define an 'allele' and (b) how to name each allele [(25)]. Obviously, the larger the gene, the larger the number of SNPs expected to be found. For genes spanning >100 kb, for example, the recombination fraction will increase, and such a definition of alleles and carrying out linkage disequilibrium studies become increasingly problematic.

Table 1: Basics definitions and terms used in Pharmacogenomics

Important definitions
A gene is a Physical and functional unit of heredity, which carries information from one generation to the next. In molecular terms, it is the DNA sequence necessary for the production of a functional protein or RNA.
Genotype is the entire construction of an individual cell or organism. Phenotype is the observable characteristics of a cell or organism.
Occasionally, one of the nucleotides in a DNA sequence may change. This is known as single nucleotide polymorphism (SNP).
All genes exist at the same location on two homologous chromosomes. The two forms of the gene are known as alleles. If the two alleles are identical, the person is homozygous for that gene. If the two alleles differ, the person is heterozygous for that gene.
Pharmacogenetics-the study of interindividual variations in DNA sequence related to drug response.
Pharmacogenomics: the study of the variability of the expression of individual genes relevant to disease susceptibility as well as drug response at cellular, tissue, individual or population level (the term is broadly applicable to drug design, discovery and clinical development).
A pharmacogenetic test has been defined by the Nuffield council on Bioethics as "a test to detect the presence or absence of, or change in, a particular gene or chromosome in order to predict a person's response to a medicine". The test can be done directly (by analyzing a person's DNA) or indirectly, by examining DNA products, such as proteins.

Role of the Pharmacist

The introduction of pharmacogenetic testing could alter prescribing practice. In current practice a doctor examines the patient, makes a diagnosis and prescribes a medicine. Pharmacists ensure that the medicine has been prescribed appropriately. Occasionally, they may also be involved in arrangement that will become more frequent as the numbers of pharmacist prescribers (both supplementary and independent) increase [(43- 47)].

Pharmacogenetic testing has the potential to add several steps to this process. Once a diagnosis has been made and the decision taken to start drug therapy, the prescriber will need to determine if pharmacogenetic testing would aid drug or dose selection. If so, informed consent and a blood sample or buccal swab will need to be obtained from the patient [(43-47)]. In addition, test result will require interpretation to determine the appropriate drug and dose, and the patient may need to be counseled about the implications of the result. It is possible that pharmacist could be responsible for some or all of these additional stages, along with having a role in monitoring and reviewing treatment.

It has been suggested that for pharmacists to integrate pharmacogenetics into their practice, they will need to

1. Clinically appraise evidence and acquire relevant knowledge.
2. Provide relevant counselling and obtain patient consent.
3. Obtain, handle and test patient samples (and maintain appropriate records).

4. Interpret test results and communicate these to patients, along with their wider implications, effectively.
5. Prescribe appropriate treatment
6. Work as part of a team-solve problems, make decisions and refer when necessary.
7. Develop diagnostic and disease management skills.
8. Apply risk managements.

It is thought that pharmacist will also be involved in educating the public and other health professionals. Both the department of Health and Royal Pharmaceutical Society has declared support for developing the role of the pharmacist in this way.

At present, only a small number of pharmacists in highly specialized roles (eg, those providing specialist HIV services) are involved in pharmacogenetics. However, several pharmacy stakeholders believe that more pharmacists may become involved in future as pharmacogenetics permeates a greater number of clinical areas [43-47].

With the development of pharmacogenetic technology still in its infancy, the primary profession should be looking to improve the education of its members. Rather than being taught how to deliver a service, pharmacists need to develop a fundamental understanding of this technology, so that they can suggest areas of clinical practice for which it could provide maximum benefit to patients.

There has been much hype surrounding the benefit that pharmacogenetics may deliver to patient care, much of which is unsubstantiated. However in defined clinical areas, pharmacogenetic testing may help pharmacists to improve pharmaceutical care.

Pharmacists need to have access to appropriate education and training in pharmacogenetic testing. This will allow them to keep pace with developments that may affect their practice and avoid becoming passive spectators of the expansion of knowledge in this field.

Consideration of ethnic differences

For most candidate gene studies, it should be feasible to use relatively small numbers of patients. For many years now, the NIH has encouraged and strongly urged the inclusion of minorities and ethnic groups in any clinical study. Interestingly, it was recently estimated that the European admixture in African Americans living in the USA is about 0.26 ± 0.02 [48], whereas the sub-Saharan African shows much less admixture [49]. This degree of admixture creates unwanted 'genetic noise', or 'background noise', that is undesirable in carrying out any 'clean' genetic study. In the future, it should be realized that particular pharmacogenetic phenotypes – e.g., rapid acetylator versus slow acetylator traits – might represent more well defined and distinct populations than particular racial or ethnic populations that virtually always exhibit admixture.

As mentioned above, genotyping as few as 5 or 10 individuals [36, 50] can lead to difficulties as to not knowing whether a particular 'singleton' is an example of micro heterogeneity or a *bona fide* informative cSNP or pSNP. The Halushka et al. [32] report is an excellent example of a study adequately covering two ethnic groups – 28 Mb (190 kb from 148 alleles) looking for cSNPs and pSNPs in unrelated individuals of African and European descent. Ideally, it would be best to screen equal numbers (35–40 each) of unrelated individuals of (a) northern European, (b) sub-Saharan African, (c) Chinese Asian, and (d) native-American descent; this (140–160 alleles from four very different

ethnic groups) would represent a 'sound' number of alleles >100 and would be about the most highly informative ethnic study. Although it would be ideal to strive for this type of multiethnic study, obviously this would be difficult for the average pharmacist. The vast majority of common cSNP and pSNP alleles will occur in heterozygotes [32–37], and the issues of phenotypic variation should be addressed in such a group of heterozygotes.
Because of ethical considerations, it is being recommended that all information – including ethnic background – be excluded from DNA samples used in pharmacogenetic and molecular epidemiologic studies. Given the extreme ethnic diversity of pharmacogenetic differences [6, 14, 20], however, it is obvious that withholding such data will impede progress in these research fields.

Quantitative trait loci (QTL) mapping

Within the next 1–2 years, SNPs located every 3–50 kb will have been characterized, thus offering the possibility of a total genome scan at high resolution. This has been termed QTL mapping [30, 51], and is regarded here as beyond the scope of this review. Hence, it will soon become possible to define extreme populations having an unequivocal phenotype (as described above), and then to screen individuals of both populations with a highly refined SNP map of the entire genome. This will offer information about major genes that contribute to a particular disease, or pharmacogenetic difference, as well as 'modifier', or secondary, genes that also affect the trait.

DMEs and individual risk of cancer and toxicity

Until a few years ago, it was commonly believed that one particular gene would be responsible for a given disease. We now realize that virtually all human diseases will represent the combined manifestation of 'major' genes and 'modifier' genes. For example, earlier in this decade, *BRCA*1 was declared by some to be 'the gene' for breast cancer; after the discovery of *BRCA*2 and further careful epidemiological studies, most investigators now conclude there may be as many as 4–12 'major' genes and an additional dozen or more 'modifier' genes responsible for increased risk of breast cancer. DME genes and DME receptor and transporter genes are clearly among the modifying factors that will affect enhanced risk of cancer susceptibility or toxicity to drugs and environmental agents. This can be caused by the accumulation of covalent binding and oxidative stress during decades of one's life. For example, the 'slow-acetylator' phenotype is associated with an increase in breast cancer risk by > 4-fold in postmenopausal cigarette-smoking women [52]; this is presumably due to the slower metabolism of reactive nitrosamines in cigarette smoke over a lifetime.

Conclusions

A test can now be developed to identify patients before they become symptomatic, which would allow them to be monitored and possibly treated using surgery. Researchers around the world identify more and more genes and genetic variations with a role in a wide variety of diseases and disorders. Announcement such as susceptible gene has been discovered is on the rise and hit the headlines almost daily. With these announcements

comes hope that the discoveries could help to speed up the design of treatments. What the researchers has created, however, is a complex web of legal, social and ethical problems for researchers, patients and physicians.

Should a patient be tested for a disease for which there is no treatment? What if someone is found to be susceptible to a complex disease, such as hypertension, lung cancer or diabetics; will, can and should that imply that he or she has to modify his or her lifestyle? Must a patient inform his or her family of genetic test result? What, if anything, might change a physician's responsibility to keep a patient's information confidential? Should drugs be sold only to people with specific genotypes? Moreover the possibility of genetic discrimination in the workplace and in health insurance, social stigmatization, and the implications for public health and the physician-patient relationship are all issues raised by genetics based healthcare. Few definitive answers have not been found for any of them, nor have laws been changed yet.

Genetic testing might well complicate the physician-patient relationship, because although the physician must already report infectious diseases to public health authorities, the question arises whether they should also report risks found by the test-not necessarily a genetic test-to relatives in danger. Such questions of patient privacy and confidentiality versus public and family health have not yet been resolved and it is not clear whether new laws will be an effective solution. Thus, the pharmacist, as well as the medical genetics counselor, will need to keep abreast of the latest information in the fields of Pharmacogenetics and pharmacogenomics.

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