Introduction to pharmacogenetics

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Abstract

In daily clinical practice there is a considerable variability in drug response between individuals. Pharmacogenetics is the science studying the inter-individual variability in drug response resulting from genetic factors. In this context, drug response concerns therapeutic response and side effects. At the moment, the field of pharmacogenetics has mainly focused on conceptual thinking. However, in medical practice there is a call for research outcomes translated into manageable guidelines. Together with information on the genetic constitution of individual patients, these guidelines could be a useful tool for individualising drug choices and pharmacogenetic-based dose adjustment. This article is the first of a series of three focusing on the field of pharmacogenetics. We provide an introduction into basic concepts and principles, clarified with examples. The next article will focus on pharmacogenetics in clinical practice and discuss major conditions and job responsibilities. The final article concerns an inventory of the ethical implications that could arise as a result of the developments in pharmacogenetics.

Keywords

Pharmacogenetics, individualised pharmacotherapy, polymorphisms, therapeutic response, drug safety

Introduction

The ideal drug would be the one effectively treating or preventing disease without any adverse effects in every patient. Unfortunately, no such drug has been developed. It turns out that in daily clinical practice there exists considerable inter-individual variability in drug response: for one patient the drug can prove to be effective without (too many) adverse effects whereas in the next patient, the drug is effective but not well tolerated because of adverse effects, and in yet another patient, the drug is neither effective nor tolerated at all. Table 1 gives an impression of the overall efficacy of drugs for a variety of indications. Different determinants could explain this inter-individual variation in drug response. The most important determinants are: physiological patient characteristics (such as gender, age, weight and fat percentage), pathophysiological characteristics (liver or renal failure, or other concomitant morbidities), hereditary influences, environmental factors (e.g. drug interactions,

Table 1: Prevalence of responders on drug therapy

Indication	Percentage
Alzheimer's disease	30
Depression (selective serotonin reuptake inhibitors)	62
Asthma	60
Diabetes mellitus	57
Incontinence	40
Migraine (acute)	52
Migraine (prophylaxis)	50
Heart rhythm disorders	60
Cancer	25
Schizophrenia	60
Rheumatic disorder	50
Hepatitis C	47

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smoking, nutrition), the pharmaceutical quality of the drug and the way the patient uses the drug (e.g. medication compliance). The idea that differences in the human genome could explain a part of the variability in drug response emerged about 40 years ago. Because of increasing knowledge and the speed with which the field of molecular biology has developed over the past five to 10 years, the hereditary component as an explanatory factor of the inter-individual variation in drug response has been the subject of growing interest. Pharmacogenetics is the study of inter-individual variations in DNA sequence related to drug response [1]. In this context, drug response involves therapeutic response and side effects.

History

The study of pharmacogenetics originated in the mid-20th century. In those days, primaquine-induced haemolysis was associated with glucose-6-phosphate dehydrogenase (G6PD) deficiency. In this deficiency, the pentose phosphate cascade in erythrocytes is blocked, resulting in a reduction in the synthesis of reduced glutathione. Reduced glutathione protects erythrocytes against several drug-induced oxidation reactions, thereby preventing haemolysis. A decrease in the availability of reduced gluthation increases the risk of haemolysis, especially in the presence of certain drugs such as primaquine.

Before technology allowed the determination of individual genetic variation, pharmacogenetics was mainly based on gross ethnic variation. Primaquine-induced haemolysis was particularly prominent among African Americans and people originating from the Mediterranean area with G6PD deficiency and diagnosed by means of enzymatic assays [2, 3].

With the introduction of the polymerase chain reaction (PCR), isolation of individual genetic variations became possible. One of the first examples was the discovery of different subtypes of the enzyme N-acetyl transferase-2 (NAT-2); this is a phase-II enzyme that is relevant in the metabolic pathway of the anti-tuberculosis drug, isoniazid [4]. In some patients, known as "slow acetylators", sustained high plasma levels of isoniazid with a "normal" dosage causes peripheral neuropathy and liver toxicity. The difference in isoniazid-metabolising capacity between normal acetylators and slow acetylators was found to be the result of differences in base sequence within the DNA segment encoding for the synthesis of NAT-2.

Polymorphisms

The human genome contains all the hereditary information and is encoded in DNA- embedded macromolecules called chromosomes. Each human cell contains a total of 23 pairs of large linear nuclear chromosomes, giving a total diploid number of 46 per cell (23 originating from the father and 23 from the mother). DNA includes both a functional and a non-coding sequence (99% of the human DNA is not functional, as far as we know). The functional part of DNA, which codes for the synthesis of a protein, is called a gene. A difference in base sequence within DNA is referred to as a mutation. Most mutations are clinically irrelevant because they do not lie within the functional part of DNA. The field of pharmacogenetics is only concerned with mutations affecting gene function. Because of mutations, several variations called alleles, exist for each gene. If mutant alleles are prevalent in more than 1% of the normal population, they are called polymorphisms. Because of this, mutant alleles, as a consequence of spontaneous mutations, are excluded from the definition "polymorphism". The most elementary polymorphism is called a single nucleotide polymorphism (SNP [pronounced SNIP]). SNPs are single mutations that differ by only one base pair from the most prevalent allele called "wild type". The wild type, which is also indicated in nomenclature, is given the suffix "*1". Variant alleles are indicated with the suffixes *2, *3, *4, etc.; depending on the succession of their discovery.

Many alleles are known for the liver enzyme CYP2D6, which is involved in the metabolism of several psychotropic drugs. Table 2 displays the relative activity of some common alleles of CYP2D6. Single nucleotide mutations can result in altered protein function or even complete loss of function. CYP2D6*1 and CYP2D6*2 display normal activity, CYP2D6*9 and CYP2D6*10 have reduced activity, and CYP2D6*3 and CYP2D6*4 have no functional activity. Another variant of polymorphism concerns the gene duplication presenting two or more copies of the wild type or variant alleles in one DNA coil. Gene duplication results in increased expression of the same gene, resulting in increased protein formation. In nomenclature,

Useful websites for further information
1. Human genome project information. Pharmacogenomics medicine and the new genetics. www.ornl.gov/hgmis/medicine/pharma.html (accessed 3 July 2007).
2. National Human Genome Research Institute. www.genome.gov/ (accessed 3 July 2007).
3. The Pharmacogenomics and Pharmacogenetics Knowledge Base. www.pharmgkb.org/ (accessed 4 July 2007)
4. Human Cytochrome P450 (CYP) Allele Nomenclature Committee www.imm.ki.se/CYPalleles/ (accessed 3 July 2007).
5. The National Center for Biotechnology Information (NCBI) . dbSNP database (nomenclature and nucleotide numbering at the genomic level according to guidelines of the Human Genome Variation Society. www.ncbi.nlm.nih.gov/projects/SNP/ (accessed 4 July 2007)

Table 2: CYP2D6 alleles	
Allele	**Enzyme activity**
CYP2D6*1	Normal activity
CYP2D6*2	Normal activity
CYP2D6*3	No activity
CYP2D6*4	No activity
CYP2D6*5	No activity
CYP2D6*6	No activity
CYP2D6*9	Less activity
CYP2D6*10	Less activity
CYP2D6*1x2	Increased activity
CYP2D6*2x2	Increased activity
CYP2D6*4x2	No activity

gene duplication is indicated with the suffix "xn" where "n" represents the number of duplications. For CYP2D6, several gene duplications have been discovered (Table 2). Gene duplication of the wild type (CYP2D6*1x2) results in increased enzyme activity. On the other hand, gene duplication of inactive enzymes (CYP2D6*4x2) has no effect on enzyme activity.

Effect on drug response

Pharmacogenetics focuses in particular on polymorphisms encoding for:

1. proteins affecting pharmacokinetic parameters (drug-metabolising enzymes or transporter proteins)
2. proteins affecting pharmacodynamic parameters (receptors or ion channels)
3. proteins affecting the pathogenesis of disease.

Possible consequences of polymorphisms on drug response concern either increased or decreased therapeutic efficacy or drug safety (adverse drug reactions and drug-drug interactions). Table 3 displays a variety of practical examples illustrating several different possibilities.

These proteins are discussed below.

Pharmacokinetic polymorphisms

Pharmacokinetics is the science that studies drug handling by the body: absorption, distribution and metabolism of drugs. Until now, most studies in the field of pharmacogenetics have focused on pharmacokinetic polymorphisms. The result of more activity, altered activity or no activity of drug-metabolising enzymes as a consequence of polymorphisms, in genes encoding for these enzymes, largely influences inter-individual differences in exposure to these drugs.

The metabolising enzymes thiopurine S-methyltransferase (TPMT) and inosine triphosphatase (ITPA) have been shown to be most important in the metabolism of the purine antagonists azathioprine and 6-mercaptopurine. Results from recent studies in patients suffering from either Crohn's disease or ulcerative colitis have demonstrated that the relative risk for life-threatening leukopaenia in patients using thiopurines was respectively 6.32 (95% confidence interval (CI): 2.14-18.63) and 3.50 (95% CI: 1.12-10.97) in carriers of a variant allele for TPMT and ITPA respectively, compared with non-carriers of these polymorphisms [5].

Patients suffering from rheumatoid arthritis are known to respond differently to methotrexate (MTX). MTX efficacy is thought to be the result of inhibition of the folate cycle, including among other factors, inhibition of methylenetetrahydrofolate reductase (MTHFR). Carriers of MTHFR 1298AA and MTHFR 677CC (wild types for two different alleles) have been found to have an increased chance of a good response to MTX with a relative risk of 2.3 (95% CI: 1.18-4.41) compared with carriers of mutant alleles [6].

Concomitant use of two drugs both being substrates for the same enzyme, or concomitant use of two drugs of which one is an inhibitor or an inducer for the enzyme involved in the metabolism of the other, possibly results in a potentially clinically relevant drug-drug interaction. In carriers of polymorphisms of enzymes involved in drug metabolism, the risk of such clinically relevant drug-drug interactions could be different depending on the type of polymorphism. For example, the antimycotic agent terbinafine is known to be able to influence the plasma level of nortriptyline by inhibition of CYP2D6 activity resulting in a decrease in nortriptyline metabolism [7]. The effect of a drug-drug interaction could be more pronounced in carriers of a polymorphism of the gene encoding for the CYP2D6 isoenzyme which decreases CYP2D6 activity. On the other hand, in carriers of gene duplications for active CYP2D6 isoenzymes, an increased activity of CYP2D6 can result in a less relevant drug-drug interaction [8]. The exposure to different

Table 3: Examples of genetic polymorphisms in relation to drug response			
	Pharmacokinetics	**Pharmacodynamics**	**Pathogenesis**
Therapeutic response	MTHFR & MTX P-glycoprotein and HIV-medication	CEPT and pravastatin	Her2-receptor and trastuzumab
Drug safety (adverse effects/ interactions)	TPMT & AZA/6-MP CYP2D6 and nortriptyline	5-HT_{2C}-receptor and antipsychotics	Factor V Leiden and oral contraceptives

drugs at a "standard" dose in carriers of polymorphic CYP P-450 iso-enzymes can display a large variability compared with non-carriers of such polymorphisms resulting in alterations to efficacy or the risk of adverse events [9].

The exposure to drugs can also be influenced by genetic polymorphisms encoding for transporter proteins such as P-glycoprotein (P-gp). P-gp activity is largely dependent on genetic variation within the MDR-1 gene. Results of research in HIV-infected patients have demonstrated that in those treated with the antiretroviral agents (efavirens and nelfinavir) the increase in the number of CD4+-cells and the decrease in viral load depends on MDR-1 genotype [10].

Pharmacodynamic polymorphisms

The efficacy or adverse effects induced by a drug are often the result of binding of this drug to a target protein being either a receptor, an enzyme or an ion-channel. Polymorphisms in genes encoding for these target proteins can lead to either up- or down-regulation, or a change, such as proteins folding (thereby influencing their structure). As a consequence, when patients are given the "standard" dosage, differences in the magnitude of a drug effect between carriers and non-carriers of different polymorphisms are observed.

A well-known example of a dynamic polymorphism influencing drug efficacy is the cholesterol ester transfer protein (CETP). CETP has an important role in the transportation of cholesterol to the liver. Research has demonstrated that in carriers of a specific polymorphism in the CETP gene, the use of pravastatin showed no efficacy in preventing the risk for the primary end-point progression of coronary artery disease compared with those not on this drug therapy [11]. It is possible that in the near future, polymorphisms could be taken into account in the decision whether or not to treat individuals with a statin [12].

Blockage of the serotonin-2c receptor is associated with weight gain in patients using antipsychotic drugs [13], and increases the risk for the development of cardiovascular morbidity and mortality [14]. Different polymorphisms in the gene encoding for the serotonin-2c receptor are known, and these polymorphisms are associated with the development of weight gain in users of antipsychotic drugs [15]. Weight gain is an important reason for discontinuation of treatment with antipsychotic drugs.

Aetiological polymorphisms

Pharmacogenetic polymorphisms also interfere with pathological mechanisms, thereby indirectly influencing either drug efficacy or toxicity.

A well-known example is the association between the risk of development of a deep vein thrombosis (DVT) and the use of oral contraceptives in carriers and non-carriers of the Factor V Leiden mutation [1]. Carriers of the Factor V Leiden mutation have a six-fold increased risk of developing DVT and users of oral contraceptive agents are at a four-fold increased risk for the development of a DVT. Patients who carry the Factor V Leiden mutation and who are prescribed an oral contraceptive have a synergistic 30-fold increased risk of the development of a DVT. Carrying the Factor V Leiden mutation is therefore considered a contra-indication to prescribing oral contraceptive agents, especially in those patients displaying additional risk factors for the development of DVT [16].

An example of an aetiological polymorphism influencing efficacy of drugs is the expression of the gene encoding for Her2-neu. Of patients suffering from breast cancer, 15-25% have an over-expression of Her2-neu gene encoding for the Her2-neu receptor. In healthy women, Her2-neu is involved in the control and differentiation of breast cells. However, in patients suffering from breast cancer, Her2-neu stimulates the proliferation of cancer cells. Trastuzimab is a monoclonal antibody acting against the Her2-neu receptor. It increases survival in patient displaying over-expression of Her2-neu compared with chemotherapy alone [17]. It is one of the first drugs for which pharmacogenetic information is effectively used in clinical decision-making to initiate its use.

What will come next?

One could wonder about the implications of pharmacogenetics for the practising hospital pharmacist. First of all, it is most important to realise that at present, knowledge about genes represents only the tip of the iceberg. Current philosophy in the field of pharmacogenetics mainly concerns concepts. However, hospital pharmacists need to know about the practical aspects; there is a need for practical guidelines in which pharmacogenetic knowledge is translated into clinical rules. Together with information on the genetic constitution of individual patients, these rules are a promising tool for individualising drug choices and pharmacogenetic-based dose adjustment optimising balance in efficacy and adverse effects. This article, the first in a series of three focusing on the field of pharmacogenetics, provides an introduction into basic concepts and principles, clarified with examples. The next article will focus on pharmacogenetics in clinical practice and discuss major conditions and job responsibilities. The final article concerns an inventory of the ethical implications that might arise as a result of the developments in pharmacogenetics.

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