PHARMACOGENOMICS AND PHARMACOGENETICS

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Keywords: Genetic polymorphism, genotype, phenotype, pharmacokinetics, pharmacodynamics

Contents

Summary

Pharmacogenetics describes the impact of a person's genetic make-up on drug action or response. Genetic variability is a major factor contributing to inter-individual variability in drug response, toxicity and side effects. The impact of genetic variability is evident not only on drug pharmacokinetics, via altered absorption, distribution and elimination processes, but also on drug pharmacodynamics, via altered drug targets. This chapter will commence with some definitions and terminology, a brief overview of genetics and the link to medicines/drugs, a history of pharmacogenetics. It will also deal with the

role and importance that pharmacogenetics can play in the pharmacokinetics (metabolism and transport) and inherent pharmacodynamic properties of drugs. The final section will deal with the status of the clinical application of pharmacogenetics particularly to drug safety and future directions.

1. Introduction

When people take the recommended dose of a medicine, some may show no response, some a small response, some a large response and some may experience side-effects and overt-toxicity leading to drug withdrawal. This large inter-individual variation in drug response is a very common feature of medicines taken by patients. It means that some medicines should not be given to patients with certain characteristics, some patients may require low doses and some may require higher than the recommended dose in order to achieve optimal therapeutic benefit. This reflects the concept of individualized drug treatment.

It is estimated that for most medical conditions, the drugs that are available are ineffective in between 20 and 80% of patients due to lack of response or side effects. There are many factors which contribute to this variation in drug response. They include patient characteristics such as age, body weight, gender and compliance behaviour, disease characteristics such as kidney and liver diseases, and environmental factors such as smoking status, nutrition and taking other medicines, including herbal medicines. It is now recognised that for many medicines, an additional factor contributing to variation in drug response is the person's genetic makeup. This has been known for more than 50 years, indeed, thousands of years, and not only does it deal with variation in drug response but also is a major factor contributing to drug toxicity and side effects. It is considered that genetic factors made a substantial contribution to the estimated 100 000 hospitalised patients having fatal adverse drug reactions in the USA (the 6th leading cause of death in 1994).

The term pharmacogenetics was coined in 1959 by Friedrich Vogel to describe the study of genetically inherited conditions that alter the way drugs act on the body and modify the way the body acts on drugs, that is, pharmacodynamics and pharmacokinetics. With recent advances in biomedical technology and research, particularly the unravelling of the human genome, the term pharmacogenomics has entered the vocabulary to essentially mean the use of methods for identifying variations in the entire human genome, and to describe genes that contribute to a specific disease, or more importantly to a drug response (efficacy/toxicity) and to changes in gene expression. Pharmacogenomics relates to and is assessed by the genotype (see below) whereby allelic variants can affect the level of expression of genes and the expression and function of proteins that are a target for drug action (eg. G-protein coupled receptors (GPCRs), ion channels, transporters, enzymes). Pharmacogenetics is the general term that is used to explain gene-related effects on drug action and is commonly assessed by the phenotype (see below). Pharmacogenetics has evolved from not only providing greater insights and knowledge of drug action in humans, but has also contributed to a better understanding of the causes of adverse drug reactions. It is now becoming personalized through individualized pharmacogenomic monitoring which can lead to improved patient safety and improved drug treatment for diseases. It highlights

the application to the well known and practiced concept of "personalised medicine" a somewhat overused term meaning individualized drug treatment. It should be noted that not only does pharmacogenetics deal with medicines, but foods and recreational drugs such as alcohol are also implicated. Thus, pharmacogenetics can explain why people, for example, of Mediterranean descent cannot consume certain foods and why Asians are particularly sensitive to the adverse effects of alcohol.

2. How Do We Determine a Person's Phenotype?

Phenotype refers to the observed characteristics associated with a gene (eg blue eyes). A person's drug metabolism *phenotype* can be classified into one of four categories: *poor* – those with no ability to metabolise; *intermediate* – those with a reduced ability to metabolise; *extensive* – those with a normal ability to metabolise; and *ultra-rapid* – those with a highly increased ability to metabolise. A common way to determine the phenotype is to administer the drug which is metabolised by a certain enzyme, for example a cytochrome P450 isoform, and then measure the amount of drug and metabolite in either a urine or blood sample. These are then used to determine the extent of metabolism or *metabolic ratio* which is calculated as the amount of metabolite divided by the amount of drug. This is a direct measure of the intrinsic clearance of the drug. It is also possible to infer a person's phenotype via measurement of a known *pharmacodynamic response* to the drug (eg blood pressure, blood clotting ratio). The *advantage* of measuring phenotype is that the extent of metabolism or enzyme function is directly known. However, the major *disadvantage* is that this function can be altered by other drugs a person may be taking or disease states such as liver disease.

3. How Do We Determine a Person's Genotype?

A person's *genotype* is determined by direct analysis of their DNA. From a blood or buccal cell or hair cell sample, DNA is isolated from the cells. The most common technique for identifying allelic variants is polymerase chain reaction (PCR) where the DNA is used as a template and regions of the gene of interest are repeatedly copied (amplified) specifically with specially designed primers matched to the nucleotide sequence. Following this amplification, the products of the PCR can be directly sequenced or undergo *restriction fragment length polymorphism* (RFLP) analysis to identify the variants. The *advantage* of genotyping is that it cannot be influenced by other drugs or disease states. However, the *disadvantage* is that the impact of a genotype on overall function in the individual is not necessarily known.

4. Nomenclature Used For Genetic Variants

As a result of the large amount of data describing variants of the genes, it has been necessary to adopt a common system of nomenclature, especially when there are a number of variants in one gene. The gene name is always first, followed by an asterisk and then a number to indicate the defining variant is being described. The number one in this position almost always refers to the wild-type sequence of the gene. Depending on the complexity of the genetic polymorphism, the number may be followed by a letter to indicate another variation in addition to the defining variant indicated by the number. An example is CYP2D6*3B, where CYP2D6 refers to the gene, 3 indicates the defining

variant is in this case a base-pair deletion at position 2549 and the letter B indicates an additional SNP at position 1749. This system is used for most genes with well-defined genetic polymorphisms, eg drug metabolising enzymes (cytochrome P450 (CYP450) and UDP-glucuronosyltransferases (UGTs) and transporters (ABCB1). Individuals can then be classified for example as homozygous wild-type (CYP2C9*1/*1), heterozygous variant (CYP2C9*1/*2*)* and homozygous variant (CYP2C9*2/*2). However, for less well defined genes often the SNP is identified individually with the nucleotide of the wild-type sequence followed by the position number of the base-pair and then the nucleotide of the variant sequence. An example is the A118G SNP of the mu opioid receptor gene, where adenine is the nucleotide in the wild-type sequence at position 118 and guanine is the nucleotide in the variant. This can also be written with the number of the base-pair first followed by the nucleotide of the wild-type sequence, a greater than symbol and then the nucleotide of the variant sequence, 118A>G.

5. History of Pharmacogenetics and Genomics

Pharmacogenetics can be traced back more than 2,500 years ago to Pythagoras, the Greek mathematician and philosopher, who reported on life threatening haemolysis in people from the Mediterranean area consuming the broad bean (*Vicia faba*). The condition was eventually called favism, and was due to a deficiency in the enzyme glucose-6-phosphate-dehydrogenase (G6PD). This deficiency results in instability of red blood cell haemoglobin so that when substances are taken (such as the broad bean or antimalaria drugs) which cause oxidative stress to haemoglobin, G6PD cannot regenerate reduced glutathione that protects haemoglobin and haemolysis ensues. This deficiency is one of the most common genetic deficiencies in the world, affecting almost half a billion people of mainly Mediterranean, African and Asian origin.

In the 1930s, researchers identified a common genetic polymorphism which was associated with ethnicity and human response to a chemical; the lack of bitterness to phenylthiocarbamide (PTC) in 30% of Caucasian but less than 10% of African Americans. The investigators were able to determine the inheritance pattern as autosomal (not linked to sex chromosome 23) recessive (need 2 copies of variant to express the phenotype). Three events in the 1950s brought pharmacogenetics to the forefront. These were: up to 10% of African American soldiers in the Pacific region during World War II developed an active haemolytic crisis when given the anti-malarial drug primaquine, due to G6PD deficiency; the short-acting intravenous muscle-relaxant succinylcholine produced prolonged apnoea (and sometimes death) in approximately 1 in 3,000 subjects due to pseudo-cholinesterase deficiency; isoniazid a very effective treatment for tuberculosis is subject to an acetylation metabolism polymorphism so that subjects can be classified as fast and slow acetylators and this influenced adverse effects, such as peripheral neuropathy and hepatotoxicity.

In addition, a landmark review in the Journal of the American Medical Association by Arno Motulsky brought to the attention of physicians, the notion that one's inheritance might explain individual differences in the efficacy to drugs and their side effects. In the mid 1970s pharmacogenetics was reinvigorated when the sparteine-debrisoquine genetic polymorphism in CYP2D6 metabolism (see below) was identified. This discovery by two clinical pharmacologists working independently in Germany and the United

Kingdom showed that the severe side effects in people taking these medicines was due to a deficiency in the enzyme CYP2D6 and its major effect on their pharmacokinetics (see below). Since cytochrome P450 (CYP) metabolism is the major drug metabolizing enzyme system, a large number of drugs metabolised by CYP2D6 could be affected (see below). This discovery led to an important and greater understanding of drug metabolism, in terms of identifying the mechanisms for the deficiency through enzyme protein purification, cloning and expression of the enzyme and identification of the allelic variants that caused the CYP2D6 poor metabolizer phenotype. Less than 10 years later, ultra-rapid metabolizer pheno- and geno-types of CYP2D6 were identified. These discoveries in CYP2D6 genetic polymorphism were quite profound resulting in almost 3,000 publications and it had a major impact on the pharmaceutical industry that was inclined to abandon the development of any drug candidate that was a substrate for CYP2D6, much to the dismay of pharmacogenetists.

Over the past 15 years, other CYP polymorphisms (eg. 2C19, 2C9) and the NAT2 and TPMT polymorphisms have been identified and, the first journal Pharmacogenetics (now called Pharmacogenetics and Genomics) was published. Nomenclature committees for drug metabolising enzymes and allelic variants have been established, drug transporter and drug target polymorphisms identified and the US Food and Drug Administration (FDA) has released guidelines for the submission of pharmacogenetic data with new drug applications. New specific pharmacogenetic testing equipment (Amplichip) has become available and it is now well recognised in drug development, that pharmacogenetics and genomics play important roles in the development of all new drugs coming onto the market. In amongst all these discoveries, a very important concept needs to be recognised in that the frequency of the phenotype and genotype (to be discussed below) is not fixed across ethnic groups, but shows large interethnic variability. For example, the CYP2C19 poor metaboliser phenotype occurs in about 2% of Caucasians and about 20% of the Asian population, but more than 50% of the Pacific Islander populations. The CYP2D6 poor metaboliser phenotype occurs in about 8% of the Caucasian population and less than 2% of the Asian population and the major allelic variant in Caucasians is *4 whereas in Asian subjects it is *10.

6. Pharmacogenetics of Drug Metabolising Enzymes and Transporters: Pharmacokinetics

The pharmacokinetics of drugs can be altered by variability in the genes encoding drug metabolizing enzymes such as CYP450s and UGTs which change the person's ability to metabolise the drug, or in genes controlling the distribution processes of drugs around the body such as genes encoding for drug transporters for example ABCB1.

6.1 Drug Metabolizing Enzymes

Many isoforms of the cytochrome P450 enzyme family responsible for Phase I metabolism display genetic polymorphism, with the most well defined being CYP2D6, CYP2C9, CYP2C19 and CYP2B6. In addition, several Phase II metabolism enzymes are influenced by genetic variability such as isoforms of the UGT enzyme family, aldehyde dehydrogenase, N-acetyltransferase and thiopurine S-methyltransferase.

6.1.1 Genetic Basis of the Polymorphisms

6.1.1a. Phase I enzymes

CYP2D6

Since its discovery in the mid 1970's the genetic polymorphism of *CYP2D6* has been well described with over 100 allelic variants currently identified. Many of these occur rarely in a population and there are differences in the frequency of variants and hence the occurrence of poor metabolizers between ethnic populations. For example, the most common variants in a Caucasian population are the *3, *4, *5, *6 and *7 variants, whilst in Asians the *10 variant is the most common. These variants are defined by specific mutations such as single base-pair changes through to deletion of the entire gene. In addition, functional studies have shown that *3, *4, *5, *6, *7 and *8 variants result in no functional CYP2D6 enzyme, *1 and *2 retain normal levels of CYP2D6 activity, whilst *9, *10 and *41 variants result in decreased CYP2D6 activity and duplication of the CYP2D6 gene (*1xN, *2xN) and variants in the promoter region of the gene results in greatly increased CYP2D6 activity. Combinations of these variants determine the overall CYP2D6 drug metabolising phenotypes: poor metabolisers (PM) carry two non-functional variants; intermediate metabolisers (IM) carry at least one decreased function variant; extensive metabolisers (EM) carry at least one functional variant; and ultra-rapid metabolisers (UM) carry at gene duplications or promoter variants (Table 1).

Other CYP450s

Similarly to the CYP2D6 genetic polymorphism, the variants described below determine the drug metabolising phenotype separating the population into PM and EM. In addition, ethnic differences in the occurrence of PM and individual variants also exist.

CYP2B6: This gene is also highly polymorphic with 50 variants identified to date. The *6, *8, *11, *12 and *14-*16 variants result in lower CYP2B6 expression or functional activity.

CYP2C9: To date, 40 variants have been identified in this gene. The *2, *3, *5, *9, *11 and *12 variants result in decreased CYP2C9 activity and the *6 variant results in no CYP2C9 functional activity.

CYP2C19: To date, 24 variants have been identified. The *2 and *3 variants are the most common in Caucasians and result in non-functional CYP2C19.

CYP3A5: To date, 23 variants have been identified. The *3 variant is the most common in Caucasians and results in non-functional CYP3A5.

Enzyme	Variant Allele	Population	% frequency	Phenotype
CYP2D6	*1	Caucasian Ethiopian African	36 – 40 35 28 – 33	Normal activity

		American	42	
	*4	Asian	12 - 21	No activity
		Caucasian	1	
		Ethiopian	5	
		African	0.5 – 1	
	*5	American	2 – 7	No activity
		Asian	3	
		Caucasian	7	
		Ethiopian	6	
	*10	African	0.7 – 2	Decreased activity
		American	9	
		Asian	2.5 – 3.6	
		Caucasian	41 – 51	
	*1xN, *2xN	Ethiopian	1 – 5	
		African	16	Increased activity
		American	0.8 – 2.5	
		Asian	0 – 2	
		Caucasian		
		Ethiopian		
		African		
		American		
		Asian		
CYP2B6	*1	Caucasian	50	Normal activity
		Ghanaian	39	
		African	44	
		American	59 – 76	
	*6	Asian	26	Decreased activity
		Caucasian	47	
		Ghanaian	33	
		African	16 – 18	
		American		
		Asian		
CYP2C9	*1	Caucasian	74	Normal activity
		Ethiopian	94	
		African	95	
		American	98	
	*2	Asian	14	Decreased activity
		Caucasian	4	
		Ethiopian	0	
		African	0	
	*3	American	11	
		Asian	2	Decreased activity
		Caucasian	0.8	
		Ethiopian	2	
		African		
		American		
		Asian		
CYP2C19	*1	Caucasian	87	Normal activity

		Ethiopian	85	
		African	75	
		American	54 – 67	
	*2	Asian	13	No activity
		Caucasian	4	
		Ethiopian	25	
		African	23 – 39	
	*3	American	0	No activity
		Asian	1	
		Caucasian	0	
		Ethiopian	6 - 10	
		African		
		American		
		Asian		
CYP3A5	*1	Caucasian	3 – 5	Normal activity
		Zimbabwean	0	
		African	7	
		American	0	
	*3	Asian	70 – 93	No activity
		Caucasian	78	
		Zimbabwean	27 – 50	
		African	75	
	*6	American	0	No activity
		Asian	22	
		Caucasian	13	
		Zimbabwean	0	
		African		
		American		
		Asian		

Table 1. The frequency of the major variant alleles of CYP450 drug metabolising enzymes across major ethnic groups and the resultant phenotype

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Biographical Sketches

Professor Andrew Somogyi is a pharmacist and a clinical and experimental pharmacologist. He received his PhD from the University of Sydney and had a postdoctoral position in the Department of Medicine, University of Bonn in Germany. He is currently Professor in Clinical and Experimental Pharmacology at the University of Adelaide (Australia) and Visiting Clinical Pharmacologist at the Royal Adelaide Hospital. He has over 200 publications in the area of pharmacokinetics, drug metabolism, pharmacogenetics and pharmacogenomics with specific reference to the opioid class of drugs. He is a member of the International Union of Basic and Clinical Pharmacology Sub-committee on Pharmacogenomics and a member of the editorial boards of Pharmacogenetics & Genomics and the British Journal of Clinical Pharmacology. He receives significant research funding from the National Health and Medical Research Council of Australia and provides a specialised genotyping service for contract research as a consultant to several companies. He has established a pharmacogenomic diagnostic service at the Royal Adelaide Hospital to cater for the increasing demand for medicine-related genetic testing.

Dr Janet Coller is a Post-doctoral Research Fellow in pharmacology at the University of Adelaide, Adelaide, Australia. She completed her PhD on the pharmacogenetics of drug metabolism in 2000 at the University of Adelaide and was subsequently awarded an NHMRC CJ Martin Post-doctoral Training Fellowship from 2000 through to 2004. During the first two years of her post-doctoral fellowship she studied at the Dr Margarete Fischer-Bosch-Institute of Clinical Pharmacology, Stuttgart, Germany under the supervision of Prof M Eichelbaum (a world-leader in pharmacogenetics), investigating the influence of pharmacogenetics of the cytochrome P450 enzymes on the metabolism of tamoxifen, a widely used drug for the treatment of breast cancer. Since returning to Adelaide and the University of Adelaide in 2002 her research has expanded her research interests to include pharmacogenetics of drug transporters, receptors and signalling pathways, with the common goal of translating research outcomes into clinical settings. She has given numerous local, national and international presentations, and was an Australasian Society of Clinical and Experimental Pharmacologists and Toxicologists (2002) and Australasian Pharmaceutical Science Association (2004) New Investigator speaker. Most recently she was awarded a 2007-2008 Young Tall Poppy Science Award in South Australia, Australia in recognition of her achievements and ability to communicate scientific outcomes to the wider community.