

Chapter 4. Pharmacogenetics and Pharmacogenomics

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I. INTRODUCTORY REMARKS

It has been recognized for more than 50 years that genetic differences between people contribute to interindividual differences in the response to many commonly used drugs. *Pharmacogenetics* is the term used for more than 40 years to denote the science about how heritability affects the response to drugs. *Pharmacogenomics* is an apparently new science about how the systematic identification of all the human genes, their products, interindividual variation, intraindividual variation in expression and function over time may be used both to predict the right treatment in individual patients and to design new drugs. The term pharmacogenomics was coined in connection with the human genome project, but there is no internationally accepted consensus depicting any semantic differences between pharmacogenetics and pharmacogenomics, and in practice the two terms are used interchangeably. However it seems that most would use pharmacogenetics to depict the study of single genes and their effects on interindividual differences in drug metabolising enzymes, and pharmacogenomics to depict the study of not just single genes but the functions and interactions of all genes in the genome in the overall variability of drug response, whether this is caused by pharmacokinetics, pharmacodynamics or both.

The human genome is composed of 3.1 billion nucleotide bases, and the number of genes is about 26.000. Alternative splicing is relatively common and it may add to the complexity of the human proteome. The average gene consists of 3000 bases, but sizes vary greatly, with the largest known human gene being dystrophin at 2.4 million bases. The functions are unknown for over 50% of discovered genes. Another characteristic of the human genome is that by chance two unrelated subjects have approximately 99.9 % DNA sequence homology. Still this leaves room for more than 1 million nucleotide bases being different between two randomly selected subjects. Very often the variability in DNA consists in only one nucleotide base change, and if this occurs in more than 100 subjects at a given position in the DNA, it is referred to as a single nucleotide polymorphism, abbreviated SNP and pronounced “snip”. SNPs make up about 90% of all human genetic variation and it occurs at every 100 to 300 bases. Two of every three SNPs involve the replacement of cytosine (C) with thymine (T), and SNPs can occur in both coding (gene) and noncoding regions of the genome. Many SNPs have no effect on cell, organ or whole body function, but some could predispose people to disease or influence their response to a drug.

In the classical pharmacogenetics a genetic polymorphism was a monogenic or Mendelian inherited pharmacological trait existing in at least two phenotypes (and presumably in at least two genotypes) the rarest of which exist in at least 1% of the population. The difference between the classical and the modern definition of a genetic polymorphism is among others, that a phenotype caused by homozygosity of an SNP with a frequency of 1 % has a frequency in the population of one in 10.000.

Most of the classical pharmacogenetic entities have been discovered initially through an adverse drug event in a single or just a few subjects. This was followed by speculations into the putative pharmacological mechanism and subsequently into the identification of the target protein and eventually the target gene. By sequencing the target gene from subjects with different phenotypes functionally important SNPs could be discovered, and this would lead to a genotype test and eventually the application of genotyping in clinical practice. The modern pharmacogenomic approach is to reverse the sequence and originate from genotypes or haplotypes and subsequently to study if this in any way is related to differences in either efficacy or adverse drug events in individual subjects in regular clinical studies. To summarize, classical pharmacogenetics has searched the gene(s) for an abnormal drug response of proven clinical value whereas pharmacogenomics presently and in the future will search the bearing if any, on drug response of known genes, SNPs or haplotypes.

During the past 50 years pharmacogenetics has focussed on drug metabolizing enzymes, and there are a number of reasons why this is so. Firstly, drug metabolizing enzymes may vary more in function and expression than most other pharmacokinetic or pharmacodynamic targets, the reason being, that drug

metabolising enzymes as a general rule are not per se related to health or survival. Secondly, provided that the drug is administered intravenously or absorbed completely following oral ingestion which is true for most but not all drugs) the area under the plasma concentration versus time curve (AUC) or the mean steady-state concentration of the drug is a very specific marker of drug elimination, ie. a low concentration means rapid elimination and a high plasma concentration means slow elimination. If in turn the elimination of drug depends on one enzyme, then the drug level is a very specific in vivo marker of the enzyme in question. Finally, the experimental setup, the drug assay technology and enzyme kinetics and pharmacokinetics applied in both in vitro and in vivo drug metabolism research is very much the same irrespective of what drug metabolizing enzyme or what drug is under study, and that creates a big flexibility in research.

While modern pharmacogenomics still is a science in the making, the pharmacogenetics of drug metabolising enzymes and particular the cytochrome P450 (CYP) enzymes has been in the focus for almost 30 years. This remainder of this chapter deals with in vivo methodologies and strategies in CYP pharmacogenetics.

II. CYP PHARMACOGENETICS

II.1. Cytochrome P450

Most drugs are lipophilic compounds that are mainly eliminated by oxidation catalyzed by the cytochrome P450 (CYP) enzyme system in the liver. The total number *CYP* genes in all species is 270, but the human CYP superfamily consists of 57 *CYP* genes and 33 pseudogenes organized in 18 families and 42 subfamilies. The CYP play a key role in the metabolism, and the endogenous substrates for CYP include fatty acids, eicosanoids, sterols and steroids, bile acids, vitamin D, retinoids and uroporphyrins. The CYP also represent the most important way for detoxification of many foreign chemical including drugs. The drug metabolizing CYP belong to families 1, 2 and 3.

II.2. CYP2D6

The CYP2D6 is the source of the sparteine/debrisoquine oxidation polymorphism, and in 7-9 % of Caucasian populations referred to as poor metabolizers the enzyme is not expressed due to mutations in the *CYP2D6* gene on the long arm of chromosome 22. The frequency of poor metabolizers in Blacks and Orientals is only about 2-3%. *CYP2D6* displays a marked allelic heterogeneity, and approximately 80 known variants, mainly in the form of single nucleotide polymorphisms in the gene, have been reported. However, the *CYP2D6**3, *4 and *5 together predict about 90% of poor metabolizers. The CYP2D6 oxidizes approximately 60 drugs including all of the tricyclic antidepressants, some antipsychotics, selective serotonin reuptake inhibitors, antiarrhythmics, beta-adrenoceptor blockers and opiates.

Separation of individuals into the extensive and poor metabolizer phenotype can be done by either phenotyping or genotyping, and each approach has advantages and disadvantages. The classical model drugs for studying CYP2D6 are sparteine and debrisoquine, and they still are superior to all other probe drugs in this regard. The reason is that they both are almost exclusively oxidized by CYP2D6 and the amount of metabolite appearing in the urine reflects CYP2D6 very precisely, provided urine collection has been complete. The second important feature is that the fraction of the two drugs not metabolized is excreted unchanged in substantial amounts via the kidneys. Thus the metabolic ratios debrisoquine/4-hydroxydebrisoquine and sparteine/dehydrosparteines in 8-12 urinary samples following oral ingestion of 10 and 100 mg debrisoquine and sparteine, respectively, provide accurate and very specific measures of CYP2D6 irrespective of any urine loss. The distribution of both metabolic ratios is clearly bimodal, and extensive and poor metabolizers thus are clearly separated. Poor metabolizers have debrisoquine and sparteine metabolic ratios above 12.6 and 20 respectively (antimodes), and extensive metabolizers have

metabolic ratios below these values. However both sparteine and debrisoquine are obsolete drugs and hence no longer manufactured. Dextromethorphan is an alternative, and a dextromethorphan/dextrorphan ratio above 0.3 in most population studies has indicated the poor metabolizer phenotype. The problem with most substrates of CYP2D6 apart from sparteine and debrisoquine, in terms of serving as probe drugs, is that in the absence of CYP2D6 their preferred route of elimination is still oxidation via the same route to the same metabolite as that made by CYP2D6 only this being catalyzed by alternatively low affinity CYPs and at a much slower rate. The alternative CYPs also vary in activity, and although the parent compound/metabolite ratio in plasma or urine indeed reflects CYP2D6 very accurately, it does not separate extensive and poor metabolizers completely as do sparteine and debrisoquine metabolic ratios.

In Caucasians approximately 1% carries one or several extra copies of *CYP2D6* and these individuals are always ultrarapid metabolizers. However only about 15% of phenotypically rapid metabolizers arbitrarily defined as subjects that have a metabolic ratio of sparteine below 0.15 have the duplicated allele. The molecular mechanism of ultrarapid metabolism in the remainder 85% is not known.

During the last 20 years the pharmaceutical industry has largely banned the development of CYP2D6 substrates because of the difficulties in handling a polymorphically metabolized drug. However there was a time where it was not common or possible to use *in vitro* methods (see chapter XX) to detect which CYPs catalyzed a particular drug including CYP2D6. There are three different *in vivo* methods that can be applied in order to find out if a drug is metabolized by CYP2D6. The phenotyped panel approach implies that the drug in question is given to 6-12 healthy extensive metabolizers and a similar number of poor metabolizers for either sparteine or debrisoquine. The drug under investigation is usually administered as a single oral dose, but sometimes it may be necessary to give it repeatedly in order to measure the steady-state concentration. CYP2D6 substrates are characterized by the fact that the AUC or C_{ss} is higher, usually 2-5 times higher in the poor metabolizers compared with the extensive metabolizers and that the total drug clearance is similarly lower in the former compared with the latter. Usually, but not always, the elimination half-life is 2-5 times longer in the poor compared with the extensive metabolizers. An alternative approach is to investigate a randomly selected group of typically 20-30 patients receiving the drug in question for treatment and to correlate their steady-state concentrations with the sparteine, debrisoquine or dextromethorphan metabolic ratios. For CYP2D6 the correlation is positive, i.e. the higher the steady-state concentration the higher the metabolic ratio. The third method is based on the use of a selective potent inhibitor and here quinidine has been the most commonly used. Six to twelve healthy extensive metabolizers take the drug either once or repeatedly and AUC or C_{ss} is determined before and during the concomitant intake of quinidine 100-200 mg/day. For a typical CYP2D6 substrate either of these pharmacokinetic parameters increases 2 to 5 times during quinidine. Quinidine is preferred over other inhibitors of CYP2D6 because it is the only one that selectively inhibits CYP2D6 and not other CYPs. The method can be refined by including a group of poor metabolizers as a negative control in whom the plasma concentration does not change during quinidine. Of the three methods, the quinidine inhibition method is the least commonly used because of the risk of proarrhythmias caused by quinidine.

All three methods can be refined to look at individual pathways by detecting partial formation clearances of drugs in relation to the CYP2D6 phenotype. Research not only has identified most of the CYP2D6 substrates but it has also shown that CYP2D6 polymorphism displays marked dose dependent kinetics for most of its substrates and that it is the source of many important drug-drug interactions when two substrates that both are metabolized by CYP2D6 are co-administered.

II.3. CYP2C19

Approximately 20 years ago a genetic polymorphism in drug oxidation different from the sparteine/debrisoquine polymorphism was discovered through a bimodal distribution of the aromatic 4-hydroxylation of the (S)-enantiomer of the antiepileptic drug mephenytoin. In Caucasians 2-3% are poor

metabolizers. The S-mephenytoin oxidation polymorphism displays marked interethnic variability as 15-20 % of Orientals are poor metabolizers. About 10 years ago it was reported that the CYP2C19 is the source of the S-mephenytoin oxidation polymorphism. *CYP2C19* also displays a considerable allelic heterogeneity, and so far 9 different single nucleotide polymorphisms have been reported in the poor metabolizers. However, the *CYP2C19*2* and the *CYP2C19*3* mutations reflecting G→A SNPs in exon 5 and 4 respectively still account for approximately 90 % of the poor metabolizers. The CYP2C19 is an important mediator of the biotransformation of tertiary amine tricyclic antidepressants (N-demethylation of amitriptyline, clomipramine, imipramine and trimipramine), all of the proton pump inhibitors, the bioactivation of proguanil, moclobemide and several other drugs.

Mephenytoin is the classical model drug used for phenotyping, but the drug is no longer in clinical use and hence it is no longer manufactured. However it is still available for pharmacogenetic research. Following the ingestion of a single oral dose of mephenytoin, urine should be collected for up to 8 or 12 hours. Urine is analysed for (R)- and (S)-mephenytoin, and the ratio between the chromatographic peak areas, the S/R ratio, is determined. The stereoselective metabolism of mephenytoin is abolished in the poor metabolizers and hence the S/R ratio is close to unity. In extensive metabolizers, (S)-mephenytoin is rapidly hydroxylated and (R)-mephenytoin is slowly demethylated. Thus the S/R ratio is less than one in extensive metabolizers and usually is less than 0.1. An acid labile metabolite is formed in extensive metabolizers and this is gradually converted to (S)-mephenytoin even if the urine is kept at -20°C. Thus the longer the sample is kept before the assay the higher the S/R ratio. If the urine sample is treated with acid then the S/R ratio becomes much higher in extensive metabolizers but it does not change in the poor metabolizers. In all samples where the initial S/R ratio is determined to be above 0.5 it is advisable to add acid and repeat the assay. In the poor metabolizers the S/R ratio does not change whereas in the extensive metabolizers it increases by a factor 10 or more.

There is certainly a need for a better model drug than mephenytoin, and omeprazole appears to be a candidate. Following a single oral dose of 20 mg of omeprazole a blood sample is drawn after 3 hours. The omeprazole/5-hydroxyomeprazole ratio in plasma has been reported to be above 7 in the poor metabolizers and below 5 in the extensive metabolizers.

The *in vivo* methods for determining if a drug is metabolized are not different from what has been described above for CYP2D6 except that the inhibitor method is not used. Drugs such as fluvoxamine and moclobemide are potent inhibitors of CYP2C19 but they are not selective for this CYP. Thus an increase in plasma concentration of a drug during concomitant intake of either of these drugs does not prove that the drug in question is metabolized by CYP2C19.

II.4. CYP2C9

CYP2C9 is a major enzyme catalyzing the biotransformation of warfarin, phenytoin, fluvastatin, several NSAIDs, tolbutamide and other oral antidiabetics. The CYP2C9 also is the source of a genetic polymorphism but contrary to the CYP2D6 and CYP2C19 polymorphism this was not discovered through a bimodal distribution of a metabolic ratio for one of the drugs metabolized by the enzyme. Rather it was discovered by sequencing of the gene and detection of several SNPs.

*CYP2C9*1* is the wild-type allele, and besides there are two important single nucleotide polymorphisms the *CYP2C9*2* associated with a functionally important Arg144Cys substitution and the *CYP2C9*3* associated with another important Ile359Leu substitution. In Caucasians the frequencies of the 6 different genotypes are 65-70 %, 15-20 % and 8-10 % for *CYP2C9*1/*1*, *CYP2C9*1/*2* and *CYP2C9*1/*3*. The poor metabolizer genotypes *CYP2C9*2/*2*, *CYP2C9*3/*3* and *CYP2C9*2/*3* each occur in about 1-2%.

Tolbutamide has been proposed to be a candidate for a model drug to probe for CYP2C9, but it is not possible to separate the 6 common genotypes completely. In one clinical study by Scordo et al. (1) the average maintenance dose of warfarin [mg/week (\pm s.d.)] to achieve the desired International Normalized Ratio (INR) (a measure of anticoagulant effect) was: *CYP2C9*1/*1*: 39 (\pm 15), *CYP2C9*1/*2*: 28 (\pm 11), *CYP2C9*1/*3*: 21 (\pm 7), *CYP2C9*2/*2*: 21 (\pm 4), *CYP2C9*2/*3*: 18 (\pm 9) and *CYP2C9*3/*3*: 9 (\pm 4). Although this study differs in that it does not measure the pharmacokinetics but rather the dose required in different genotypes to achieve a desired pharmacodynamic end point, it is still a paradigm for studying the clinical relevance of pharmacogenetics in drug metabolism. It clearly shows that the CYP2C9 oxidation polymorphism is an important determinant of the warfarin dose, but it also shows that within each genotype (pheno-) type there is a considerable variability caused by other genes, the constitution of the patient and the environment. *CYP2C9* genotyping before treatment with warfarin probably has limited, if any, value in practice.

II.5. Pheno- or genotyping for drug metabolizing enzymes

Pheno- or genotyping as an aid for choosing the optimal dose from the start in theory should be performed if the drug is exclusively metabolized by a single CYP, if the drug has a low therapeutic index and if clinical dose titration is not feasible. For CYP2D6 the possible candidates include tricyclic antidepressants, some antipsychotics, and some antiarrhythmics and for CYP2C9 the possible candidates could be warfarin and phenytoin. However pheno- or genotyping for CYP enzymes has never achieved widespread use in clinical practice because, as explained above, the response is not determined alone by a single enzyme or gene.

The advantage of phenotyping compared with genotyping is, that phenotyping gives an up-to-the-minute account of the CYP in question integrating the result of the genetic, constitutional and environmental influences. The disadvantage is that it involves the ingestion of a model drug which is often obsolete as a therapeutic agent and hence no longer manufactured and also that it is necessary to collect urine or to draw a blood sample. Genotyping separates patients into categories, it is not influenced by environmental or constitutional factors and it only has to be performed once in the life time of the patient.

III. FUTURE DIRECTIONS

On the basis of the last 25 years of intensive studying the pharmacogenomics/-genetics of CYP enzymes a number of general statements regarding the role of genetic factors for variation in drug response can probably be formulated. First until proven otherwise the response to any drug is always determined to some extent by genetic factors. However drug response is never determined by a single gene or by a group of genes alone. It is rather determined by several interacting genes and with important influences from the environment and from the constitution of the patient.

Now that the human genome is known in practically all detail there is widespread optimism, that in the near future, it will be possible to tailor the treatment to the individual patient on the basis of the patient's genotype. This author does not entirely share this optimism. Genotyping as an aid to select the right dose of the right drug in the individual patient would be of theoretical use if the response is mainly determined by a single gene or a limited group of genes, and if all of the environmental and constitutional influences have a more limited influence, and besides are known in detail and can be measured in the individual patient. It is anticipated that less than 10 % of drugs in 10 years from now will be prescribed following a pharmacogenetic test. However by extrapolation from the 25 or more years of experience in the CYP field studying pharmacogenetics/-genomics will lead to new important insights and discoveries that will ultimately lead to the development of new and better drugs and to the rational use of drugs that are already on the market. According to this author's view it is here that the true importance and benefits of pharmacogenetics lies.

IV. REFERENCES

1. Scordo MG, Pengo V, Spina E, Dahl ML, Gusella M, Padrini R. Influence of CYP2C9 and CYP2C19 genetic polymorphisms on warfarin maintenance dose and metabolic clearance. Clin Pharmacol Ther 2002;72:702-710.

This chapter was written on the basis of numerous studies that have not been cited in the text. As a textbook that gives a comprehensive overview and is reasonably well updated the following can be recommended:

2. Levy RH, Thummel KE, Trager WF, Hansten PD, Eichelbaum M, eds. Metabolic Drug Interactions. Philadelphia: Lippincott Williams & Wilkins, 2000.
There is a website with a constant update and comprehensive overview:
3. Oscarsson M., Ingelman-Sundberg M., Daly A.K., Nebert D.W. (Eds.). Human cytochrome P450 (CYP). Available at <http://www.imm.ki.se/CYPalleles>.