Cytochrome P450 Part 2: Genetics of Inter-Individual Variability

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ABSTRACT

Inter-individual variability in drug metabolism results from the influence of a myriad of factors, such as concomitant drug therapy and genetic factors. Advances in recombinant DNA technology have enhanced our understanding of the extent of genetic variation in the cytochrome P450 (CYP) enzyme super family, thus clarifying the molecular basis of many clinically observed variations in drug response. This second article in the CYP series describes current understanding of genetic variability in the major drug metabolising CYP enzymes, nomenclature used to describe variant *CYP* genes, and the clinical significance of such variability.

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INTRODUCTION

Inter-individual response to chemical exposure – therapeutic, environmental or occupational – is a well-documented phenomenon with a myriad of underlying causes including age, health, gender, nutritional status, concomitant drug therapy and genetic factors. The importance of genetic differences in the metabolism of drugs and environmental chemicals has been recognised for at least four decades.[1,2] Historically, genetic variation in drug metabolism was usually identified following unexpected responses to standard doses of therapeutic drugs.

Genetic variability in drug metabolism is evident when the frequency of an appropriate measure of *in vivo* enzyme activity (e.g. urinary metabolic ratio) is polymodal, most commonly bimodal. In bimodal distribution, the two phenotypes are generally termed extensive and poor metabolisers or fast and slow metabolisers. In some instances, additional phenotypes such as the ultrarapid or intermediate metabolisers, can be detected, resulting in additional modes within the distribution (Figure 1). The underlying cause of polymodal phenotypes can be genetic variability within the genes encoding for drug metabolising enzymes.

TYPES OF GENETIC VARIABILITY

The types of genetic variation that influence cytochrome P450 (CYP) activity range from a change involving a single base in a given gene through to major chromosomal alterations such as the deletion or duplication of an entire gene. The most common type of genetic variation described is the single nucleotide polymorphism, that involves the substitution of one nucleotide for another at a precise location within the genome. Closely related to single nucleotide polymorphisms are the indels – insertion or deletion of one or more nucleotides from the gene. The impact of single nucleotide polymorphisms and indels on CYP enzymatic activity is governed by where in the genome these variations occur and by the nature of the genetic change

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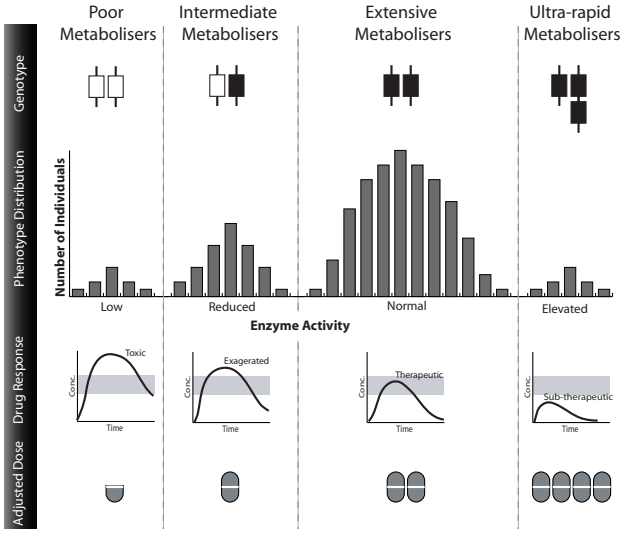

Figure 1. Relationship between genetic variation, enzyme activity, drug response and optimised drug dosing. Genetic variation as indicated by genotype (white boxes = defective allele, black boxes = function allele) can produce four different levels of enzyme activity (phenotype).

that occurs. Single nucleotide polymorphisms within coding regions may change a single amino acid in the protein product of the gene, possibly altering its activity. While indels frequently result in major structural changes in the protein that can have deleterious effects on enzyme activity.

Variation outside of coding regions, including regions previously thought to have no significant functions, may impact on the regulation of gene-altering protein levels. Frequently, variants will result in reduced enzymatic activity although notable exceptions, resulting in significantly increased activity do exist. Each distinct variant of the gene is referred to as an allele, for example if a gene contains one single nucleotide polymorphism there will be two alleles. The most common allele is referred to as the wild-type while the less common (minor allele) is referred to as the mutant. To be considered a polymorphism, the minor allele must occur in the population with a frequency of over 1%, however, the term polymorphism is frequently used to describe any genetic variation.

Allelic variation in many *CYP* genes is well documented, with the high number of identified alleles necessitating the formation of an official human *CYP* allele nomenclature committee and web site.[3] To name *CYP* alleles, the gene name and allele are separated by an asterisk followed by Arabic numerals. The wild-type allele is always assigned the number 1 with variants assigned in chronological order of their identification – *CYP2D6*1* is the wild-type *CYP2D6* allele while *CYP2D6*4* is the third identified variant. For many *CYP* genes it is possible to have more than one variant per copy of gene. When one variant present within the gene is the major determinant of enzyme activity and the other variants are only minor differences thought to be of no functional significance, the alleles are assigned the same numerical designate but are differentiated by a letter (e.g. *CYP2D6*3A* and *CYP2D6*3B* share a common variant that determines enzyme activity but also contain additional separate variants of limited significance).

The clinical implications of genetic variability in *CYP* genes are dependent on the function of the encoded enzyme. Given the diversity of CYP substrates, the differences in *CYP* genes may impact on many aspects of human health, ranging from drug and chemical sensitivities to cancer. For genes that encode CYP enzymes with critical physiological roles, defective alleles may be catastrophic. In contrast, allelic variability in *CYP* genes which encode drug metabolising enzymes does not directly cause pathology but may increase an individual's sensitivity to the harmful effects of drugs and chemicals. Many genetically variant CYP enzymes also metabolise non-drug chemicals (e.g. environmental pollutants) linked with human diseases such as cancer. Given this role in the metabolism of chemical toxins, much attention has focused on the relationship between the *CYP* genotype, the resultant phenotype (enzyme activity) and susceptibility to chemical-induced diseases.[4]

With respect to drug metabolism, the clinical relevance of genetic variability in *CYP* genes is dependent on many factors, such as the alleles present, patient's clinical state, therapeutic index of the drug, smoking status and concomitant drugs. As many drugs are metabolised by multiple CYP enzymes, the percentage of total drug metabolised by a genetically variant CYP is also a major consideration.

ASSESSING GENETIC VARIABILITY

From a clinical perspective, the aim is to predict a patient's response to a given dose of a particular drug. In part, this requires an assessment of the individual's metabolic capacity. With respect to the *CYP* genes this may be achieved in two ways. The first technique, genotyping, investigates the patient's DNA to determine which specific alleles are present. The second approach, phenotyping, assesses a specific CYP enzyme's level of activity.

Genotyping provides information about which *CYP* alleles are present and the level of enzyme activity is then inferred from the genotype. This requires the association between a given genotype and the resultant phenotype to be clearly understood. For some genetic variants, this relationship has not been definitively established rendering them of limited use for predicting drug dosage requirement. Recent advances in molecular biology have yielded significant improvements in genotyping techniques but no single approach has established itself as being significantly superior to all others. Perhaps the most notable technique, with respect to the CYP enzymes, is the US Food and Drug Administration-approved Roche Amplichip. This platform, based on micro-array technology, enables an individual to be genotyped for twenty-nine CYP2D6 and two CYP2C19 polymorphisms in a single test. The ability to genotype multiple alleles simultaneously is an important advance as it reduces testing costs and improves result turnaround times. In a clinical setting, genotyping is minimally invasive needing only a small blood sample or cheek scraping to yield sufficient DNA for analysis.

Phenotyping involves the collection of urine after the administration of a probe substrate and determination of a relevant metabolic ratio. For example, poor metabolisers would have a much lower ratio of metabolite to parent drug in urine. By contrast, phenotyping provides a direct measure of enzyme activity but does not determine the source of the variability, such as genetic factors or consumption of enzyme inhibitory drugs. Each CYP to be phenotyped requires a separate probe substrate. Ideally, the probe should be metabolised by a single enzyme, be non-toxic and inexpensive, e.g. dextromethorphan is commonly used for phenotyping CYP2D6. In a similar vein to the Amplichip, it is possible to phenotype multiple CYP enzymes at one time through the administration of a 'phenotyping cocktail' containing a probe substrate for each of the major drug metabolising CYP enzymes. Phenotyping is cumbersome as patients are required to consume a probe substrate followed by specimen collection and sample analysis.

GENETIC VARIABILITY

Humans possess around 50 functional *CYP* genes.[5] Despite this multiplicity, the bulk of drug metabolism is catalysed by a small number of CYP enzymes found in families 1, 2 and 3. The predominant drug-metabolising enzymes are CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4. Although quantitatively a minor enzyme in terms of drug metabolism, CYP2A6 metabolises specific substrates.

CYP1A2

CYP1A2 metabolises a number of drugs, such as theophylline, caffeine, clozapine and olanzapine. In addition, the human CYP1A2 enzyme accommodates a plethora of toxicologically important substrates including food-derived mutagens and products of combustion processes. Levels of CYP1A2 are increased by exposure to certain chemicals including many inhaled during the smoking of cigarettes and cigars. Significant inter-individual variability in human CYP1A2 enzyme levels and/or activity has been observed but the precise phenotypic impact of genetic variability within *CYP1A2* has not been completely elucidated.[6-9] Several variants have been identified within the coding region, however, they tend to have a negligible impact on enzyme activity or occur at very low frequencies. Despite the limited significance of these variants it is likely that genetic variability located within the regulatory regions of the *CYP1A2* gene contributes to the observed variability in CYP1A2 levels. It has been demonstrated that variants within this region influence the degree of induction caused by tobacco consumption.[10,11] Patients with at least one of the *CYP1A2*1D* or *CYP1A2*1F* alleles experience a relatively greater increase in CYP1A2 levels than other genotypes. In patients who do not smoke, the presence of these alleles does not result in altered CYP1A2 expression. While these variants explain some of the variability in CYP1A2 activity that is observed it is likely that variants within other genes, which directly interact with CYP1A2, will also be important.

CYP2A6

The human CYP2A family comprises three genes in a single cluster – *CYP2A6*, *CYP2A7*, *CYP2A13*. Although CYP2A6 is the major CYP2A enzyme, its role in drug metabolism is quantitatively small. However, it is important in the metabolism of coumarin and nicotine.[12] In addition to the wild-type *CYP2A6* allele, three defective *CYP2A6* alleles have been identified which result in an absence of CYP2A6.[3] While defective CYP2A6 metabolism is of limited relevance with respect to drug metabolism, the relationship between CYP2A6, nicotine metabolism and smoking behaviour may be relevant. Of interest is the relationship between defective *CYP2A6* alleles, smoking behaviour and cigarette consumption.[12]

CYP2C9

Although more than thirty *CYP2C9* alleles have been identified, only two – *CYP2C9*2*, *CYP2C9*3* – are of clinical significance. Around 30% of Caucasians have at least one of these alleles. When compared with the wild-type allele, the enzymatic activities of *CYP2C9*2* and *CYP2C9*3* alleles are reduced to approximately 50% and 10% respectively.[13,14]

CYP2C9 metabolises approximately 10% of all drugs and is the primary CYP involved in the metabolism of a number of clinically important drugs including phenytoin and *S*-warfarin.

The impact of the *CYP2C9* alleles on warfarin therapy has been extensively studied. While CYP2C9 is not the sole determinant of warfarin sensitivity, individuals with defective *CYP2C9* alleles require lower doses, take longer to achieve a stable maintenance dose and are at an increased risk for over-anticoagulation and serious bleeding events.[15-17]

CYP2C19

CYP2C19 genetic variability was first identified as a result of variability in the metabolism of S-mephenytoin.[18] Although more than 20 alleles have been defined only two alleles – *CYP2C19*2*, *CYP2C19*3* – are responsible for the majority of poor metabolisers. Around 5% of Caucasians and 20% of Asians will carry two of these defective alleles.[19] A promoter region variant, *CYP2C19*17*, has been identified which confers an elevated CYP2C19 protein level and enhanced metabolic activity.[20] While CYP2C19 is involved in the metabolism of a number of clinically important drugs, such as omeprazole, diazepam, sertraline and citalopram, the therapeutic significance of its polymorphisms remain controversial. The low clinical attention to these alleles can be attributed to several factors. Firstly, many of the drugs metabolised predominantly by CYP2C19, such as the proton pump inhibitors, have relatively large therapeutic indices that limit the toxicity associated with poor metaboliser status. Secondly, for some substrates, such as sertraline and citalopram, the dose-effect relationship is not well defined. Despite these issues, CYP2C19 polymorphisms can be of clinical significance, for example, poor metaboliser phenotype is associated with enhanced *Helicobacter pylori* eradication rates relative to extensive metabolisers following treatment with standard doses of proton pump inhibitor containing triple therapy.[21] On this basis it might be anticipated that homozygotes for the *CYP2C19*17* allele, who experience a one-third reduction in proton pump inhibitor exposure, would experience a lower cure rate but no such effect has been observed.[22] The difference in eradication success rates between extensive and poor metabolisers has prompted suggestions that proton pump inhibitor dosage should be adjusted for *CYP2C19* metaboliser status.

CYP2D6

CYP2D6, with over 60 alleles described, represents the most extensively studied example of genetic polymorphism in drug metabolism. These alleles can be divided into four main categories – null, decreased, normal and increased activity – that result in four phenotypes – poor, intermediate, extensive and ultrarapid metabolisers. A patient's phenotype is determined by the most active of their alleles — 5 to 10% of Caucasians and 1% of Asians are poor metabolisers while 1% of Caucasians and 29% of black Ethiopians are ultrarapid metabolisers.[23]

Of all the alleles that have been identified, several are notable because of their importance to specific phenotypes. *CYP2D6*4* is the most common Caucasian null allele, with a frequency of 20%, and accounts for 90% of poor metabolisers.[24] The *CYP2D6*4* allele contains a single nucleotide polymorphism that results in major structural defects within the protein rendering it devoid of activity. The poor metaboliser phenotype is produced by the presence of two null alleles. *CYP2D6*10* is an allele of decreased activity and has a very high frequency in Oriental populations, exceeding 50%, but only 2% in Caucasians.[25] An individual heterozygous for a null allele and a decreased function allele (e.g. genotype **4/*10*) has the intermediate metaboliser phenotype. In contrast, an individual that is heterozygous for a decreased function allele and a normal function allele (e.g. genotype **10/*1* or **10/*2*) is or homozygous for the normal function alleles (e.g. genotype **1/*1* or **1/*2*) is classified as an extensive metaboliser. The final alleles of significance are those of the gene duplications that are responsible for the ultrarapid metaboliser phenotype.[26] Alleles carrying as many as 13 functional copies of CYP2D6 have been identified. A duplication allele is indicated by the notation *xN* where *N* represents the number of gene copies.[25]

The therapeutic relevance of variability in the *CYP2D6* gene arises due to the large number of clinically important drugs metabolised by CYP2D6 combined with the narrow therapeutic index of many of these drugs. CYP2D6 substrates are represented in many different therapeutic classes such as beta-blockers, anti-arrhythmics, psycholeptics and antidepressants. In most clinical situations, standard doses of CYP2D6 substrates may result in elevated blood levels in poor metabolisers, increasing the risk of toxicity. Important exceptions to this general rule are pro-drugs such as codeine, which require CYP2D6 catalysed activation, where poor metabolisers may not achieve appropriate analgesia.[26] At the other end of the phenotypic spectrum, ultrarapid metabolisers are generally considered to be a at increased risk of therapeutic failure. Once again pro-drugs are the exception to this rule, where ultrarapid metabolisers may experience toxicity. This is illustrated by the fatality of a breastfed 13-day-old neonate which was attributed to opioid toxicity resulting from maternal ingestion of 30 mg of codeine twice a day.[27] The mother's codeine dose had been reduced from 60 mg twice a day as a result of somnolence and constipation after two days of treatment.[27]

The clinical relevance of the *CYP2D6* polymorphism is best illustrated by the clinical resurgence of the anti-anginal, perhexiline.[28] Originally, discarded due to hepatotoxicity and neurotoxicity associated with its use, perhexiline is considered useful in patients who are poorly controlled or are refractory to conventional drug regimens. As perhexiline toxicity is more prevalent in patients with the *CYP2D6* poor metaboliser phenotype, safe use of the drug can be achieved by adjusting the dose according to an individual's metabolic capacity.[29] The dose adjustment strategy employed clinically uses a form of phenotyping where the metabolism of perhexiline itself is used to provide a measure of CYP2D6 activity.[30]

CYP2E1

CYP2E1 is toxicologically important as it metabolises paracetamol and many carcinogens. CYP2E1 levels are elevated following exposure to alcohol. Seven *CYP2E1* human alleles have been identified including mutations in structural and regulatory regions of the gene.[3] Only one of the variant *CYP2E1* alleles *(CYP2E1*2)* appears to result in compromised metabolic function towards CYP2E1 substrates.[31] While genetic variability in the *CYP2E1* gene has been linked with altered susceptibility to several chemical-induced cancers, the therapeutic relevance in terms of drug metabolism remains unclear.

CYP3A4

The human CYP3A family comprises two genes expressed in adults – *CYP3A4*, *CYP3A5* – and a third gene – *CYP3A7*, which is expressed only during fetal life. While *CYP3A5* plays a minor role in drug metabolism, CYP3A4 is a major drug metabolising enzyme, metabolising around 60% of drugs to some extent. CYP3A4 is also the predominant CYP in human liver where it comprises up to 60% of total CYP content and is also the major CYP in the human intestine.[32,33]

Although no evidence exists for a bimodal distribution of CYP3A4 activity, the repeated observation of wide inter-individual variability in CYP3A4 activity has prompted close scrutiny of the *CYP3A4* gene. To date, more than 20 alleles have been described, several of which result in reduced enzyme activity. However, due to the very low frequency of these alleles they do not account for the observed variability in enzyme

activity. Given the high degree of inducibility of CYP3A4, it is likely that genetic variation within as yet unidentified regulatory regions or other interacting genes is partly responsible for the differences in enzyme activity observed in the population.

CONCLUSION

The last decade has provided a great deal of data on genetic variability in *CYP* genes, through the identification of variant alleles and resultant insights into the molecular basis of observed metabolic phenotypes. Further analysis of regulatory regions of *CYP* genes will enhance our understanding of why some CYP enzymes exhibit profound inter-individual variability. Any gene that encodes a protein involved in the response to an administered drug, is potentially variable. This vast array of proteins includes all drug-metabolising enzymes, drug receptors, drug transporters and proteins involved in the pathophysiology of the disease being treated. The term applied to the field of genetically determined drug response is pharmacogenomics. Theoretically, the ability to accurately predict drug response on the basis of prior genetic knowledge has profound implications for rational therapeutics and drug development. Armed with such knowledge it should be possible to better predict patients likely to be at risk of drug toxicity. Cost savings can be achieved in drug development through better clinical trial design and early dismissal of drugs of genetic concern.

The burgeoning pharmacogenomic field provides many sobering ethical and economic challenges for future therapeutics. If we are to embrace pharmacogenomic knowledge into mainstream therapeutic practice, there will be a need for non-invasive technology enabling high throughput genotyping. Recent progress in this area has been rapid with the manufacture and marketing of the sophisticated 'gene chips', suitable for *CYP2D6* and *CYP2C19* genotyping on a broad scale. Although not in widespread clinical use, gene chips have the potential to bring pharmacogenomics to the clinic or pharmacy. Can we expect 'home genotyping' kits driven by nano technology in the next decade or so? The answer most likely lies in economics rather than technological capability.[33] For example, an interesting development would be technologies that genotype many polymorphic sites (even the whole genome). This would only need to be done once, then the data could be reused as required. This would have to circumvent the issues of turnaround time for genotyping as well as issues of cost-effectiveness – it may not be cost-effective to undertake a specific genotype for a specific drug, but if the genotype is available then it is probably cost-effective to use it.[34] Regardless, a balanced appreciation of the role genetic variability plays in determining individual drug response is essential for best therapeutic practice.

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