Commentary

Genetic Polymorphisms, Drug Metabolism and Drug Concentrations

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The interfaces between genetics and drug metabolism have recently been the subjects of intense research activity. Pharmacogenomics uses molecular biological techniques to study genes in relation to drug therapy for specific diseases in order to identify new treatments. Pharmacogenetics investigates the genetic basis for differences in individual responses to drugs with regard to their metabolism and transport in the body. A genetic polymorphism occurs if, within a population, a single gene responsible for producing a metabolising enzyme has a variant allele with the arbitrary frequency of 1%.[1] For many such genes single nucleotide polymorphisms (SNP) exist and an allelic site may have more than one SNP. Genotype is the detailed gene structure of an individual whereas the more commonly measured phenotype is the outcome of metabolism of a drug in an individual. Since phenotype is the result of interactions between genetic make-up and the environment it is not always concordant with genotype.

Drug metabolism is conventionally described as consisting of phase 1 oxidation reactions, primarily mediated by cytochrome P450 enzymes in the liver, and phase 2 conjugations such as glucuronidation, sulphation and acetylation. The first drug metabolising polymorphism was described over 40 years ago for acetylation. It is now known that N-acetyltransferase (NAT) is controlled by two genes (NAT1 and NAT2) of which NAT2 A and B are responsible for clinically significant metabolic polymorphisms.[2] Many common drugs such as caffeine, isoniazid, nitrazepam and sulphonamides are acetylated. If isoniazid or another marker drug is given and plasma or urine drug concentration measured after a standard time interval it is possible to separate individuals into one of two groups: fast acetylators who have only low concentrations of parent drug remaining and slow acetylators who have much higher concentrations.[3] Long before modern day techniques were available for identifying genes it was demonstrated by phenotyping studies in twins and in different ethnic groups that these differences were under genetic control. Caucasian and Negro populations have approximately equal proportions of fast and slow acetylators whereas oriental groups have almost 90% fast acetylators.[4] Variable drug concentrations can produce a variety of clinical outcomes. Slow aceylators have more side effects (e.g. dapsone and procainamide) and fast acetylators have less reliable clinical responses with drugs (e.g. isoniazid). Fast Acetylators also have the potential for more side effects in situations where an active metabolite is responsible for toxicity. It is now possible to identify slow and fast acetylators by analysing NAT2 genes but this is essentially a research procedure and in routine laboratories phenotype is measured by analysing the drug/metabolite ratio in urine after standard doses of drugs such as caffeine or isoniazid. This is most commonly requested for patients with tuberculosis. Glucuronidation is also under genetic control but no clinical correlations have been described.

Polymorphisms of phase 1 cytochrome P450 (CYP450) metabolising enzymes were first described about 30 years ago when it was noted that a small proportion of subjects given the antihypertensive drug debrisoquine had extreme falls in blood pressure which were related to abnormally high plasma drug concentrations. It has subsequently been found that debrisoquine and well over 70 other drugs are metabolised by the enzyme known as CYP2D6. This is one of a series of cytochrome P450 enzymes found in the liver endoplasmic reticulum. Each of these enzymes has a unique, but often overlapping, range of drug substrates.[5] Genotype can be identified by a number of techniques but this remains a research procedure. Phenotype is measured by giving a drug known to be a 'marker' for a certain enzyme and measuring the urinary ratio of parent drug to metabolite some hours later. This divides populations into extensive metabolisers (EM) with a low ratio and poor metabolisers (PM) with a ratio more than 10- to 100-fold greater. More recently, a very small proportion of ultrarapid metabolisers (URM) with multiple

gene copies and very low urine metabolic ratios have been described.[6]

CYP2D6 remains the best investigated enzyme and it is known that EMs carry an autosomal dominant wild type gene and may be homozygous or heterozygous for this allele. PMs have two abnormal alleles and over 80 genotypic variants have been described which account for about 98% of phenotypic PMs. These include point mutations, insertions and gene deletions, which result either in no enzyme or very limited quantities of stable enzyme being produced. Depending on the nature of the allelic variation in the heterozygous EMs they may be classified as intermediate metabolisers.[7]

CYP2D6 metabolises many antidepressants, antiarrhythmic agents, beta blockers, opiates, neuroleptic agents and other drugs such as perhexiline and phenformin. Population studies have shown that approximately 8% of Caucasians but less than 1% of Asian races are PMs.[5,7] The consequences of the various polymorphisms parallel those for acetylation. PMs have higher than normal plasma drug concentrations and hence an increased incidence of adverse drug reactions (ADRs).[1,8] They also have an absence of metabolites that can be very significant if, as in the case of codeine that is converted to morphine, an active metabolite is responsible for therapeutic actions. PMs for CYP2D6 do not get any analgesic effect from codeine.[9] Perhexiline, an antianginal agent, produces severe and irreversible peripheral neuropathy in PMs if standard doses are used for more than a brief period.[10] It is therefore essential to perform perhexiline therapeutic drug monitoring in the early stages of therapy. Similarly, phenformin was removed from the market because it caused an unacceptably high incidence of fatal lactic acidosis which occurred mainly in the PM subgroup of the population.[11] EMs have a variable range of plasma concentrations for CYP2D6 target drugs and may also be subject to induction and inhibitory interactions caused by other drugs. Inhibition can result in "phenocopying" in which genetic EMs are turned into phenotypic PMs by agents such as major tranquillisers.[8] URMs may suffer from therapeutic inefficacy with drugs such as tricyclic antidepressants.[6]

In view of the wide range of drugs metabolised by this enzyme it is often asked whether CYP2D6 genotyping should be routinely available. In my view this is not appropriate at present. It is expensive, can be time consuming and is not cost effective. It will miss about 2% of PMs and will also fail to identify situations in which phenocopying has occurred. It is more appropriate to be aware of which drugs are metabolised by CYP2D6 (Table) and to identify, or avoid, significant ADRs by clinical monitoring of pulse rate, blood pressure and ECG. Therapeutic drug monitoring is vital for drugs such as perhexiline.

Numerous other polymorphic CYP450s have been identified (Table). The CYP2C family is particularly important. CYP2C8 has only recently been clearly separated from 2C9 and two variant alleles have been identified. The PM frequency is currently unknown but the enzyme may be induced by phenobarbitone and inhibited by ketoconazole or gemfibrozil in EMs. CYP2C9 metabolises a number of very clinically significant agents (Table).[12,13] PM frequency lies somewhere between 1 in 100 to 1 in 500. The enzyme may be inhibited by sulphaphenazole and fluconazole. Genotypic variability and drug interactions in EMs are particularly important for potentially life-saving, or life-threatening, drugs such as warfarin, sulphonylureas and phenytoin. Recent studies of CYP2C9 genotype in relation to INR have shown that the two described allelic variants may lead to reduced enzyme activity, increased warfarin concentrations and increased INR.[14] One study showed that subjects with variant alleles had a significantly increased risk of above range INRs and of serious bleeding.[15] Should we therefore genotype for 2C9 in patients going on to warfarin? At present I consider that the answer is "definitely not". Warfarin is a racemic drug and the S isomer, which is metabolised by 2C9, has the majority of effect. However R warfarin also has some action on clotting factors and is metabolised by other enzymes such as CYP1A2 and CYP3A4. In addition many other factors are involved in the final INR level including vitamin K concentrations and mutations which may be present in the clotting factors. Finally, and most importantly, it is very simple and cheap to monitor INR which is the significant outcome measurement.

The proportion of PMs for CYP2C19 has a wide variation in different ethnic groups with Caucasians having approximately 2-4% whereas South East Asians have up to 20% of PMs.[16] Eight allelic variations have been described as have a number of common drug substrates (Table). The enzyme may be inhibited by fluoxetine or ketoconazole and it has been shown that Asian populations need lower doses and are at higher risk of ADRs with drugs such as diazepam. Where the metabolite is the active agent (e.g. proguanil which is converted to cycloguanil) it is EMs who experience more ADRs. Recent studies on the eradication of *Helicobacter Pylori* using triple therapy of a proton pump inhibitor, clarithromycin and amoxycillin, have shown that eradication rates are higher in PMs than in EMs (especially homozygotes).[17]

The most abundant enzyme (more than 50% of hepatic cytochrome P450s) responsible for metabolising more than 400 drugs, is CYP3A – particularly 3A4. There is a wide range of content of this enzyme in different populations and the enzyme is subject to multiple interactions; in particular, inhibition by macrolide antibiotics and onazole antifungals. However, although one or two polymorphisms have been

Table. Some common cytochrome P450 enzymes with examples of substrates and specific inhibitory drugs.

Enzyme	Substrates	Inhibitors
CYP2D6	Beta Blockers (some pathways) Clozapine Codeine Flecainide Haloperidol Mexiletine Perhexiline Phenformin Tricyclic Antidepressants	Fluoxetine Quinidine
CYP2C8	Arachadonic Acid Paclitaxel Rosiglitazone Zopiclone	Gemfibrozil Ketoconazole
CYP2C9	NSAIDs ('profens') Phenytoin "sartans" (AII Inhibitors) Sulphonylureas Warfarin	Fluconazole Sulphaphenazole
CYP2C19	Diazepam S-mephenytoin Moclobemide Proton Pump Inhibitors Proguanil SSRIs [selective seretonin reuptake inhibitor] (some pathways)	Fluoxetine Ketoconazole

described (mainly for CYP3A5) these have not been clearly correlated with significant clinical outcomes and the enzymes seem to be predominantly under polygenic control.[18]

There are a number of other genetic polymorphisms of interest in relation to drug metabolism, the most notable of which is thiopurine methyltransferase (TPMT). This metabolises both mercaptopurine and azathioprine and 1 in 300 of Caucasians have very low enzyme activity. In children with acute lymphatic leukemia (ALL) high TPMT activity can cause poor clinical response and lack of TPMT can result in severe or fatal myelosuppression. Genotyping is therefore essential in children with ALL and is being explored in the management of Crohn's Disease.[19,20]

Other areas of growing interest include transporters such as P-glycoprotein or organic acid transporters which move drugs across membranes including the gut and the blood brain barrier.[21] Many commonly used and important drugs are carried by these transporters and may be subject to both induction and inhibitory interactions. Although some genetic polymorphisms have been identified, clinical associations have not yet been clearly defined.[22]

In summary, polymorphic drug metabolism is an important area of therapeutics with a rapidly growing body of knowledge. At present, with the exception of *in vitro* procedures for TPMT, genotyping remains a research procedure. Phenotyping should be available in large laboratories for acetylation and the best understood cytochrome P450 enzymes. The present role of the laboratory for patients taking substrate drugs remains largely in the realm of therapeutic drug monitoring for perhexiline, phenytoin, digoxin etc. and the routine measurement of INR for warfarin. These serum drug assays will remain essential and more assays are likely to be required in the future. Interpretation of results must be based on knowledge of the polymorphisms, ethnicity and drug interactions. Early hopes of prescriptions targeted by genotype have not yet been fulfilled and it is likely that genotyping will always be confined to large specialist laboratories and used only in limited situations or in clinical trials.[23]

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