Review Article

Clinical Use of Pharmacogenomic Tests in 2009

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Abstract
Pharmacogenomics is a new field where testing an individual can define either a risk status for an adverse event, or the rate of metabolism of a drug. This is achieved by the categorisation of the enzyme activity or documenting genetic polymorphisms of a metabolising enzyme. The best example of risk status assessment is the recent finding that HLA-B typing a person can predict whether they are at risk of a severe skin reaction from the drug abacavir. Those patients showing HLA-B*5701, who are being considered for abacavir therapy, can be prevented from developing potentially toxic epidermal necrosis (TEN) or Stevens-Johnson Syndrome by avoiding abacavir. The evidence for HLA-B typing for allopurinol and carbamazepine has also been described. Most other pharmacogenomic tests are of drug metabolising enzymes, which can either be assessed using "probe" drugs and measuring a ratio of parent drug to metabolite, or, by genetic testing for polymorphisms of the genes. In practice, testing is usually done by molecular testing, but this typically does not detect all polymorphisms. This article briefly reviews the evidence for the utilisation of pharmacogenomics for antidepressant drugs, tamoxifen, codeine, warfarin, azathioprine, clopidogrel, omeprazole, tacrolimus and irinotecan. There are few pharmacogenomics tests being carried out in practice, as there has not been a wide appreciation of their use, and only limited evidence exists for many individual drugs. It is expected that utilisation will increase as more evidence becomes available and there is a wider understanding of the existing evidence by the medical profession.

Introduction
Pharmacogenetics is the study of the genetic basis of drug response.[1] Pharmacogenomics is a term which is often used interchangeably, but refers to pharmacogenetics plus genomics and proteomics.[2] The recognition that most human drug responses are multifactorial has led to the realisation that personalised medicine implies a broad consideration of factors and thus has resulted in the frequent use of the broader term pharmacogenomics.[3] Hence, this article will follow the convention of using the term "pharmacogenomic (PGX) tests" for testing that influences drug response.

Some PGX tests have been acknowledged for a long time and are accepted as routine tests; e.g. it has been known that those patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency should avoid certain drugs because of the risk of haemolysis following their intake. The effect of a drug is traditionally assessed by clinical outcome, which includes laboratory tests and therapeutic drug monitoring (TDM). It has only been in the last 10–15 years that a number of PGX tests have been available to supplement the traditional outcome measures and to aid with dose determination and choice of medication. This review will discuss tests where there is evidence of clinical utility.

PGX tests can theoretically reflect any stage of the drug use cycle - absorption, distribution, metabolism and excretion. In practice, however, the tests, where there is a case for clinical use, are mainly involved with the metabolism of a drug or in the induction of an immune response. There is still not agreement about the place of PGX tests in clinical practice. To be useful, the PGX test must provide information beyond the traditional measures of clinical outcome and therapeutic drug monitoring. Not all drugs have TDM available and a PGX test needs to be done only once, whereas TDM tests may be required on a number of occasions, thereby improving its cost-effectiveness.

The involvement of an enzyme in the metabolism of the drug is not enough evidence to advocate measuring its function as

a clinical test, because it may not be possible to measure differences in function in that enzyme. In addition, even if it can be shown that the particular enzyme is not functioning, it may have little effect, as the drug may be metabolised by alternate pathways. This has recently been illustrated for the drug clopidogrel, which is an inhibitor of the platelet P2Y12 adenosine diphosphate receptor. Clopidogrel is converted to 2-oxo-clopidogrel by the cytochrome P450 enzymes CYP2C19, CYP1A2 and CYP2B6, and then to the active metabolite, known as R-130964, by CYP3A, CYP2C9, CYP2C19 and CYP2B6. Some of these enzymes can be monitored by looking at polymorphisms in their respective genes. This has been done in two recent studies in the *New England Journal of Medicine*.[4,5] In one study, carriers of at least one *CYP2C19* reduced-function allele were at significantly increased risk of death from cardiovascular events compared with non-carriers and had 32% less exposure to the active metabolite of clopidogrel than non-carriers.[4] No significant effect of any of the other CYP genotypes was found. A second study showed a similar relationship with *CYP2C19* reduced function alleles and increased cardiovascular events.[5] They also genotyped *CYP3A5* and the transporter *ABCB1*, and the receptors *P2RY12* and *ITGB3*.[5] Only *CYP2C19* and to a lesser extent, a variant in *ABCB1*, were found to have a relationship with adverse events. Both studies showed that despite the many enzymes known to be involved in the metabolism, only CYP2C19, and to a less convincing degree *ABCB1*, were found to have any justification for testing to determine efficacy.

The effect of a drug is influenced by many other factors such as co-medication, disease status and body weight. These are often factors known to the treating physician, however, the functional status of the significant drug metabolising enzyme is not known. Monitoring these through PGX tests (which separate individuals into those likely to have too high or too low blood concentrations) may add to the understanding of lack of efficacy or the occurrence of adverse drug reactions. PGX tests are more likely to be useful in treatment with those drugs with a narrow "therapeutic window".[6] Concentrations below the window are associated with lack of efficacy while concentrations above with adverse drug events.

This review will discuss PGX tests where there is evidence that variations in the amount of drug metabolising enzyme can be measured and is correlated with observed or likely clinical effects. The small number of drugs selected reflect those with most evidence and provide specific examples that illustrate the utility of these tests. Initially, an overview of PGX methods is summarised with examples followed by evidence-based test examples, where the pharmacokinetic and/or clinical effect has been shown to be significant.

There are two main methods for PGX tests - biochemical and molecular genetics.

Biochemical Tests

Biochemical Tests of Response - "Phenotyping"

These methods typically involve the patient taking a specific drug, called a probe drug, which utilises the pathway of interest.[7] Measurements are made of the parent drug and an inactive metabolite usually after eight hours. A ratio of the two is calculated - the "metabolic ratio". Patients can then be divided into categories based on their rate of drug metabolism. Normal metabolisers are called "extensive metabolisers" (EMs),[8,9] those who metabolise very slowly are called "poor metabolisers" (PMs), whilst those in-between are termed "intermediate metabolisers" (IMs). While this effect varies, PMs and IMs - if taking the standard dosage of the drug - will tend to have higher blood concentrations of the drug.[10-12]

This method of categorising the functional aspect of a drug metabolising enzyme has become known as phenotyping in the PGX literature. The particular use of the word "phenotyping", entrenched in the PGX literature, is used for any biochemical test including an enzyme assay.[7,13] Note that this is a slightly different definition of the expression "phenotype" that is used in traditional genetics.

The phenotyping (biochemical) tests are more suited to the research setting because it is logistically difficult to collect a urine specimen at eight hours in the clinical setting. The person is also exposed to possible side effects of the probe drug and therefore is required to give suitable informed consent. The probe tests can be done individually for each pathway, or given as a cocktail of drugs to measure all pathways simultaneously. These cocktails tend to be named by their place of origin such as the "Pittsburgh cocktail"[14] or the "Karolinska cocktail".[15] The issues of informed consent are even more critical for these "cocktails."

Enzyme Assays

Traditional enzyme assays are difficult for most drug metabolising enzymes as, in addition to the technical difficulties of enzyme assays, a liver sample is usually required. Sometimes a blood test can be used such as the red cell thiopurine methyltransferase (TPMT) enzyme assay (see below).

Molecular Genetics Testing

Currently most PGX tests are done by characterising the enzyme's gene to demonstrate which alleles are present. (This article follows the usual nomenclature of using capitals for the enzymes and italicised capitals for the genes.) For a number of these genes there are many different alleles (due

to the presence of polymorphisms). Some of these may be associated with loss or reduction of gene function. Alleles are denoted by an asterisk (*) and different alleles are given different numbers e.g. **1* usually corresponds to the normally functioning gene. Those patients shown to have two normal active genes correspond to the EMs, whilst PMs have two genes that are non-functional.[16,17] These phenotypes are shown for the most common genes in Table 1.

The actual effect of being a PM depends on whether the parent drug is pharmacologically active and is converted into an inactive metabolite or is a prodrug that needs to be converted to an active metabolite. When the parent drug is the pharmacologically active metabolite (Figure 1A), slow metabolism, as a PM, will lead to higher blood concentrations which may lead to adverse drug reactions. Ultrarapid metabolisers (UMs) may have such rapid metabolism that the drug cannot have any pharmacological effect. The active drug nortriptyline is converted by CYP2D6 to an inactive product and PMs will develop high blood concentrations which may be associated with an increased number of demonstrated adverse drug reactions such as drowsiness, confusion or dry mouth.[18,19]

If the parent drug is inactive, or relatively inactive, and is converted to an active metabolite, it is called a "prodrug" (Figure 1B). Codeine has relatively little pain relief itself but is converted to morphine. The effect of being a PM of CYP2D6 makes codeine ineffective as a pain reliever. UMs, on the other hand, are at risk of excess sedation and adverse drug reactions.[20,21] Additional important prodrugs are clopidogrel and tamoxifen, and are discussed below.

IMs may have one active allele and one non-active allele for some genes. In general, the enzyme activity is intermediate between PMs and EMs but the actual effect varies with the particular gene, ethnic group and drug. Thus, although the final clinical effect is complex, and the understanding of the function of the drug metabolising enzyme may only explain some of the variability in drug response, knowing the genetic status, as well as its effect on the active moiety, is an important part of understanding all drug effects and determining an appropriate dosage.

Tests of Drug Metabolising Enzymes

These are listed in approximate order of their evidence-based utility.

1. Thiopurine Methyl Transferase (TPMT)

- for thiopurine drugs e.g. azathioprine, 6-mercaptopurine.

This enzyme converts the thiopurine drug through a series of steps to the toxic thioguanine nucleotides. Only about 5% of the theoretical output of thioguanine nucleotides is needed for therapeutic action. The parent drug is broken down to less toxic products at several places in the pathway by TPMT. If TPMT is deficient, either completely as a deficient homozygote, or partially as a heterozygote, the patient is at greater risk of white cell depression including neutropaenia.

Table 1. Genotypes and their associated metaboliser phenotypes. PM, IM, EM and UM refer to metabolic activity associated with each allele. The activity of these alleles is often drug-specific and may also vary between ethnic groups. ***CYP2C9***: PM alleles are partially active alleles. The predominant alleles are *CYP2C9*2* and *CYP2C9 *3* with *CYP2C9*2* having slightly less activity than the *CYP2C9*1* wild-type (normal) allele. *CYP2C9 *3* has 10-30% activity of wild-type normal allele. ***CYP2C19*:** PM alleles are null alleles. EM alleles are functional alleles. UM alleles refers to *CYP2C19*17* which has increased activity. The UM phenotype is expected for individuals homozygous for *CYP2C19*17*. ***CYP2D6***: PM alleles are null alleles (no or little activity). IM alleles are those with reduced activity. EM alleles are those with normal activity. The IM phenotype results from IM/IM alleles or IM/PM alleles. Some literature also includes the PM/EM genotype as intermediate metabolisers but this is not widely accepted. UM alleles are those with increased activity (these are alleles carrying two or more functional genes).

Metaboliser Phenotype	***CYP2C9* genotype**	***CYP2C19* genotype**	***CYP2D6* genotype**
Poor Metaboliser	PM/PM	PM/PM	PM/PM
Intermediate Metaboliser	EM/PM	EM/PM	IM/IM, IM/PM
Extensive metaboliser	EM/EM	EM/EM	EM/EM, EM/IM, EM/PM
Ultrarapid metaboliser	None known yet	UM/UM	EM/UM, UM/UM

PM, poor metaboliser; IM, intermediate metaboliser; EM, extensive metaboliser; UM, ultrarapid metaboliser.

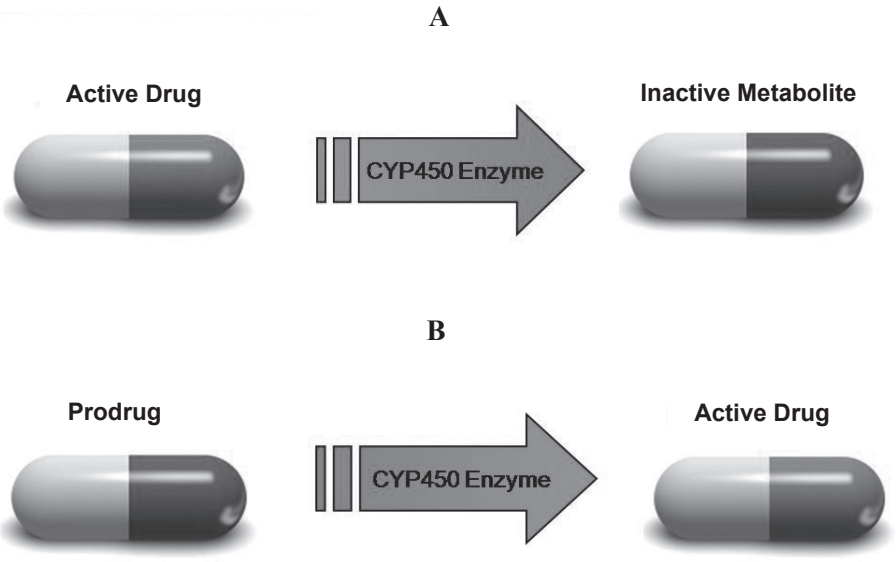

Figure 1. A, Schematic representation of the inactivation of an active drug to an inactive metabolite. The CYP450 enzyme, or one of the CYP450 enzymes that catalyses this conversion may vary in activity from individual to individual and the measurement of this is the basis of the pharmacogenomic test. Reduced function of the enzyme will tend to increase the concentrations of the active drug and possible adverse drug reactions. Increased enzyme activity may be associated with lack of drug efficacy. B, Schematic representation of activation of a prodrug which becomes converted to an active drug by the action of a CYP450 enzyme that catalyses this reaction. The CYP450 enzyme, or one of the CYP450 enzymes, that catalyses this conversion may vary in activity from individual to individual and the measurement of this is the basis of the pharmacogenomic test. Reduced function of the enzyme will lead to increase concentration of the prodrug and may lead to lack of drug efficacy. Increased function may lead to higher active drug concentrations and adverse drug reactions.

One in 300 Caucasians are deficient homozygotes and one in ten people are heterozygotes. Both homozygotes and heterozygotes require lower dosages of the thiopurine drug to avoid myelotoxicity.[22,23]

Biochemical Tests

Enzyme Assay (Phenotyping)

A direct measurement of the TPMT activity in the red cells can be carried out. It is a technically difficult assay but has the advantage that it can measure all cases of reduced function of TPMT and so can potentially diagnose heterozygotes, homozygotes, patients with low TPMT concentrations for other reasons and patients with raised TPMT concentrations. The disadvantages are that it may be inaccurate after blood transfusion, may take several weeks to get a result and there is not perfect concordance with genotyping tests.[22]

Metabolite Assay

Measurement of 6-thioguanine and 6-mercaptopurine metabolites. There is some evidence that this is an adjunct to measuring genetic status as it is a measure of dosage and is very useful when adjusting the dosage in those patients who are genetically deficient.[24]

Genotyping Tests

There are a number of different polymorphisms that can affect the function of the *TPMT* gene. In practice however, if one tests the three most common polymorphisms one can detect 80–95% of the genetic variation.[24,25] This genotyping can be done relatively quickly and is usually performed if abnormal TPMT enzyme function is found biochemically. Heterozygotes and homozygotes can be diagnosed and the dosage adjusted accordingly. The genotyping can be done faster than the biochemical test of TPMT activity and can be done from blood or a cheek brush scraping. However, it does not diagnose all *TPMT* genetically-deficient patients or those that are TPMT deficient for other reasons. It also does not diagnose high concentrations of TPMT which increase the risk of hepatotoxicity and make it less likely that the patient will respond to thiopurine drugs.

Combined Biochemical Tests and Genotyping Tests[24]

In practice, a selection of the above three tests is done depending on the experience of the particular laboratory.

Those doing TPMT biochemical assays usually follow up those beyond a conservative cut-off of low values with the genotyping test. Those primarily doing the genotyping test may also do metabolite testing and perform biochemical follow up (phenotyping) on those with high 6-thioguanine concentrations.

2. Cytochrome P450 2D6 (CYP2D6)

Biochemical Test

This is a loading test with a probe drug that is metabolised by CYP2D6. The drug is given at standard dosage and the urinary ratio of the parent drug and its metabolite is made usually eight hours after drug ingestion. The ratio of the two is called the molar urinary metabolic ratio and the actual value defines the patient's metaboliser category.[8,9] The literature discusses categorisation using drugs that are currently unavailable such as debrisoquine and sparteine, but the currently used probe drug by some laboratories is the cough suppressant dextromethorphan,[8,9] which is metabolised to the metabolite dextrorphan.

Molecular Genetics Test

CYP2D6 is a member of the very important CYP450 family and is involved in the metabolism of about 25% of drugs in clinical use. This genetic test is one of the more complicated types because there are many (>70) different alleles. Seven to ten percent of the Caucasian population have two deficient genes and are classified as PMs. There are a number of alleles that have reduced enzyme activity and the combination of an inactive and a reduced function allele is classified as an IM with reduced enzyme activity. One normal allele with an inactive allele or reduced function allele, usually functions in the normal range and would be classified as an EM. A slightly reduced function is found for some drugs. If there are more than two gene copies (3–13 have been reported), the enzyme activity is too high and the individual is called an UM.[26-28]

We will focus on only three drug examples; however, as stated earlier, CYP2D6 is involved in the metabolism of about 25% of drugs in clinical use.

<u>*Tamoxifen*</u>

Tamoxifen is used as a therapy for oestrogen-receptor (ER) positive breast cancer therapy. It is the standard of care for premenopausal women and the only hormonal agent approved for this purpose by the United States Food and Drug Administration (FDA). There is an alternative therapy for post-menopausal women (aromatase inhibitors). Tamoxifen itself interacts with ER as the parent drug but recently the metabolites of tamoxifen, endoxifen and 4-hydroxy-tamoxifen, have been found to have much more potency in preventing cell proliferation than tamoxifen. Tamoxifen requires CYP2D6 for the activation to both metabolites and also uses CYP2C19, CYP3A4 and CYP3A5. Endoxifen blood concentrations can be measured to reflect anti-tumour activity. PMs, IMs and even heterozygous EMs of CYP2D6 have all been shown to have lower endoxifen concentrations than EMs (normal).[29,30] This is also correlated with poorer treatment outcomes such as reduced relapse-free time and reduced disease-free survival. It is also correlated with more hot flushes in PMs.[29,30] This data comes largely from cross-sectional and retrospective studies but prospective studies have not yet been done. However, it suggests that *CYP2D6* genotyping could be useful in deciding about the use or non-use of tamoxifen. As there are other alternative treatments and outcome is measured in terms of years, this could be quite an important test to be considered when planning treatment.

Selective serotonin reuptake inhibitors (SSRIs) are often administered to breast cancer patients to treat depression and hot flushes. Some of these (e.g. paroxetine and fluoxetine) are potent CYP2D6 inhibitors and have the effect of effectively converting an EM to function like a PM or IM. This can be confirmed by measuring the endoxifen concentration. SSRIs are now contra-indicated with tamoxifen use. An FDA committee recently recommended re-labelling of tamoxifen to explain the effect of genetic states of *CYP2D6* on the clinical effect of tamoxifen and the interaction with SSRIs.[31]

<u>*Antidepressants*</u>

Most antidepressants are metabolised by CYP2D6 or CYP2C19 or both. Some laboratories offer both tests as an antidepressant PGX test. It has only been shown for tricyclic antidepressants that metaboliser status relates to expected blood concentrations and the increased occurrence of adverse events in PMs. However, for SSRIs, the evidence is not yet convincing in relation to adverse events, only for pharmacokinetics.[32]

Amitriptyline is a tricyclic antidepressant that is converted by CYP2C19 to the active metabolite nortriptyline, which produces the therapeutic effect, and then CYP2D6 is required for the deactivation and clearance of nortriptyline (Table 2). Adverse drug reactions tend to be associated with nortriptyline concentrations rather than with the parent drug and consequently PMs of CYP2D6 are more likely to have side-effects due to the build-up of nortriptyline in their blood.[18] These include dry mouth, blurred vision, confusion, bradycardia and reduced conscious state. CYP2C19 converts amitriptyline to nortriptyline and the rate of this will be determined by whether the person is an EM or PM.

An example of an SSRI drug is paroxetine which is metabolised by CYP2D6 and also strongly inhibits CYP2D6.

Table 2. Examples of drugs where evidence has shown PGX testing to be beneficial.

Drug	Active metabolite	Main Use	Major Gene(s) involved	Consequence of abnormal phenotype
Clopidogrel (prodrug)	R-130964	Prevent thrombosis in myocardial infarction, stroke	*CYP2C19*	Less active drug available and greater risk of cardiovascular events.
Thiopurine e.g. azathiopurine, 6-mercaptopurine	Thioguanine nucleotide (6-TGN)	Inflammatory bowel disease, childhood acute lymphoblastic leukaemia	*TPMT*	PMs have high risk of myelosuppression and neutropaenia.
Tamoxifen (prodrug)	Endoxifen (4-hydroxy-N-desmethyl-tamoxifen)	Adjuvant therapy for breast cancer to prevent recurrence	*CYP2D6*	PMs have lower blood concentrations of endoxifen and earlier relapse of breast cancer.
Amitriptyline	Nortriptyline	Depression	*CYP2D6* (and *CYP2C19)*	PMs have more adverse effects UMs are likely to have the least therapeutic response. Complicated by the involvement of CYP2C19.
Nortriptyline (active)	None relevant	Depression	*CYP2D6*	PMs have higher concentrations of unchanged nortriptyline and increased risk of side effects. UMs are less likely to have a good response when taking the standard dose.
Codeine (prodrug)	Morphine	Pain relief	*CYP2D6*	PMs are unable to convert codeine to morphine and have no pain relief. UMs have increased sedation and opioid toxicity.
Paroxetine (active)	None relevant	Depression and other mood disorders	*CYP2D6*	PMs have increased plasma concentrations of paroxetine and increased side effects. Paroxetine strongly inhibits *CYP2D6* and so may affect concentrations of other drugs that use *CYP2D6* pathways.
Warfarin (S-Warfarin)	None relevant	Prevention of clotting events	*CYP2C9* and *VKORC1 (-1639G>A, i.e. "A"* variant in promoter*)*	The presence of a PM allele of *CYP2C9* and "A" haplotype of *VKORC1* increases risk of bleeding and therefore a reduced dose is required. Inadequate dosing of normal patients may lead to clotting, stroke and heart attack.
Sertraline (active)	None relevant. Main metabolite is desmethyl-sertraline which is inactive.	Wide range of mood disorders	*CYP2C19*	PMs have accumulation of sertraline and more side effects UMs have lack of response.
Omeprazole	5-hydroxy-omeprazole	Gastric ulcers	*CYP2C19*	UMs have treatment failure. EMs require more frequent doses than PMs.
Irinotecan	SN-38	Cancer	*UGT1A1*	Individuals homozygous for *UGT1A1*28* have increased exposure to SN-38 with increased toxicity, diarrhoea, neutropaenia.

PM, poor metaboliser; EM, extensive metaboliser; UM, ultrarapid metaboliser; CYP, Cytochrome P450; UGT, UDP-glucuronosyltransferase.

PMs have higher blood concentrations than EMs (normal) although this reduces with time because the concentrations become saturated, as paroxetine is also a strong inhibitor of CYP2D6. This results in unpredictable blood concentrations. PMs may also have an increase of adverse drug reactions such as sexual dysfunction. Interactions are common with co-medication such as tamoxifen. There can be potentially fatal interactions with the non-selective, irreversible monoamine oxidase inhibitors such as phenelzine, moclobemide and tranylcypromine and a high risk of serotonin syndrome.[34,35]

Pain Relief

Codeine is used for pain relief (commonly used post-operatively) but also found in many cough and cold medicines (to prevent coughing). It is a prodrug as it does not have a pain-relieving effect itself and requires CYP2D6 to be converted to its active metabolite - morphine. Several studies have shown that CYP2D6 PMs have virtually undetectable morphine concentrations and consequently have reduced pain relief or no pain relief at all. A reduced pain relief is also apparent in IMs of CYP2D6.[36]

Meanwhile, UMs of CYP2D6 will have rapid conversion to morphine and are likely to experience increased opioid effects such as sedation and nausea. There have also been several case reports showing sedation and respiratory depression in neonates when the mother has been taking codeine while breastfeeding and some of these cases have shown that the mother is an UM of CYP2D6.[20,36]

3. Cytochrome P450 2C9 (CYP2C9)

Biochemical Test

The probe drug is losartan and the urinary product losartan /E-3174. This is usually only used in research projects where the appropriate permission to use losartan can be obtained. An alternative probe drug is tolbutamide.[37-39] Tolbutamide is currently unavailable in Australia.

Molecular Genetics Test

Warfarin is the most important drug that uses CYP2C9 as a substrate. S-warfarin, the most active form, is inactivated by CYP2C9. IMs and PMs require lower dosages of warfarin. Warfarin has a narrow therapeutic window and the patient's response is measured by the prothrombin-time international normalised ratio (INR). The vitamin K epoxide reductase enzyme (VKORC1) is required for recycling reduced vitamin K to produce vitamin K-dependent clotting factors. A variant in the promoter of *VKORC1* reduces the function of this gene and is also associated with lower warfarin requirements. Patients with abnormal *CYP2C9* alleles and the higher risk *VKORC1* genotype require a lower dose of warfarin to achieve the appropriate anticoagulant effect (stable INR), with the minimal risk of bleeding. There are many studies showing these general findings, but it is not yet known whether genotyping all patients to determine final dose significantly decreases the time to reach a stable dose and significantly reduces bleeding and other morbidity.[40,41] In order to determine this, there are randomised controlled trials being carried out in the USA and in the UK/Europe. It has been very time consuming and difficult to recruit patients for these studies. The International Warfarin Pharmacogenetics Consortium has recently shown that the use of an algorithm based on PGX testing of C*YP2C9* and *VKORC1* is significantly better than using either a clinical algorithm or a fixed dose approach.[42]

CYP2C9 is also involved in the metabolism of angiotensin-II blockers, oral hypoglycaemics and non-steroidal anti-inflammatory drugs, but testing is recommended for only some of these drugs.

4. *Cytochrome P450 2C19 (CYP2C19)*

Biochemical Test

The probe drug is omeprazole and the metabolite is 5-hydroxy-omeprazole. This test is usually only used in research.

Molecular Genetics Test

A number of drugs are substrates of CYP2C19 including some antidepressants, barbiturates and proton pump inhibitors. An UM genotype, *CYP2C19*17/*17,* has recently been described. Three important drug examples are clopidogrel, sertraline and omeprazole.

Clopidogrel is a prodrug which is converted to active form by a number of CYP450 enzymes and variants of *CYP2C19* have been found to reduce this conversion and therefore reduce the efficacy of the drug. It is not known yet if an increase in dose can overcome this problem. It is also not known if the UM genotype *CYP2C19*17/*17* would improve the function of the drug or produce an adverse drug reaction. The recent studies on clopidogrel are mentioned above.

Sertraline is an SSRI in which CYP2C19 is a major pathway.[40,43] CYP2C19 PMs have higher blood concentrations of the drug which can lead to serious adverse drug reactions such as the serotonin syndrome - confusion, muscle spasms and tremor. There is concern about agitation, suicidal thoughts and suicide especially in teenagers and young adults. It is expected that the serum concentration would be lower and the implied risk of therapeutic failure would be seen with the ultrarapid genotype *CYP2C19*17/*17* as has been shown with the SSRI drug, escitalopram.

Omeprazole is a proton pump inhibitor which is more than 80% metabolised by CYP2C19. PMs have higher blood

concentrations and as there appears to be a wide therapeutic index, adverse drug reactions are not usually seen. There is evidence that ulcers actually heal better in PMs than EMs because of the high blood level.[44] The genotype which is associated with UMs (*CYP2C19*17/*17*) may be associated with lower omeprazole concentrations and risk of treatment failure.[45,46]

5. *UDP-glucuronosyltransferase 1A1 (UGT1A1)*

UGT1A1 is the major human bilirubin-glucuronidating UGT. Homozygous defects in this gene reduce hepatic glucuronidation to a varying degree depending on the polymorphisms. Gilbert's syndrome is associated with about a 30% reduction in glucuronidation and is mostly associated with an increased number of TA nucleotide repeats in the promoter. Individuals with Gilbert's syndrome have two copies of seven TA repeats, as opposed to other individuals who have six or five. Individuals with such defects are relatively common, affecting 10–13% of Caucasians. In the past, Gilbert's syndrome was thought to be completely benign, but it is now known that the interference with glucuronidation of certain drugs may be associated with symptoms, e.g. jaundice in patients on HIV protease inhibitors such as ritonavir. A kit to look at the TA repeats in the promoter region became the first PGX test approved by the FDA in 2005. This was for patients taking the antineoplastic drug irinotecan. Those patients with Gilbert's syndrome appear to have a substantially increased risk of toxicity, manifested by myelosuppression or diarrhoea, from the active metabolite SN-38.

6. *Cytochrome P450 3A5 (CYP3A5)*

A recent trial has shown that the required dosage of tacrolimus in renal transplants is determined by the genotype of *CYP3A5*. *CYP3A5*3* is associated with lack of gene function and individuals with this variant require a lower dosage.[47]

7. *Other Tests (Not Frequently Used)*

There are some tests offered by some laboratories where the evidence is not at a level for the test to be recommended currently. These include *NAT2, CYP1A2* and *CYP3A4*. *NAT2* is involved in acetylation and there is a potential use to determine dosage for isoniazid. CYP1A2 is required for several common anti-psychotic drugs e.g. clozapine, but there is little correlation with known polymorphisms. Finally, CYP3A4 is the most abundant enzyme and is associated with metabolism of about 50% of drugs in clinical use. However, despite variability between individuals and the knowledge about polymorphisms, no convincing correlations exist between polymorphisms or blood concentrations or side effects. Biochemical probe drugs, such as midazolam, have been used to assess CYP3A4, but usually only used in research projects. Thus, for many important drug groups that are mainly metabolised by CYP3A4, there are currently no effective PGX tests. This includes calcium channel blockers and macrolides. Finally, we have not discussed at all the myriad of statistical associations published in the quest to find new PGX tests, but confined this review to genes with variants which have been shown to be associated with changes in blood concentrations, and, when known, adverse events. We also have not reviewed the new biomarkers used, especially in oncology, to determine prognosis. These are usually related to changes in the tumour tissue. An example is when demonstrating that a breast cancer is HER2 positive and this indicates susceptibility to treatment with trastuzumab (Herceptin).

Immune Reactions

There are no tests for the majority of adverse drug reactions thought to be caused by unpredictable immune reactions. However, recently there has been the discovery that the HLA-B type is a biomarker to strongly predict onset of serious skin rash (TEN or Stevens-Johnson Syndrome). There are three examples:

1. HLA-B*5701 and Abacavir Sensitivity

This finding, which originated in Australia, has now been confirmed by several observational studies and recently a randomised controlled trial.[48] Initially, it was found that the HLA-B*5701, HLA-DR7 and HLA-DQ3 haplotype was associated with the onset of the TEN/Stevens-Johnson rash.[49] The randomised controlled trial showed that HLA-B*5701 screening eliminated the occurrence of TEN/Stevens-Johnson rash, documented by an immunologically-confirmed hypersensitivity reaction. The negative predictive value was 100% and the positive predictive value 47.9%.[48] This test is the only PGX test that has its own Medicare Item in Australia.

2. HLA-B*5801 and Allopurinol-Induced Cutaneous Rash

This has been found to be a very strong association in Han Chinese and also occurs less strongly in Europeans and Japanese.[50,51] Such screening, prior to allopurinol treatment, can help prevent this potentially fatal skin reaction and allow safer medication suggesting that another drug is used.

3. HLA-B*1502 and Carbamazepine-Induced Rash

This has also been found to be very strong in Han Chinese living in Europe and Thais, but not in Europeans or Japanese.[51-53] The FDA has released a warning on the seriousness of cutaneous reaction for patients on carbamazepine and recommended HLA-B testing in patients of Asian ancestry before commencement of therapy.[54]

Drug interactions are beyond the scope of this article but often involve competition, inhibition or induction of the same drug metabolising enzymes as discussed above.

Are PGX Tests Being Used?

There are few PGX tests where the level of evidence is such that a recommendation can be made to utilise them in all patients. It is generally recommended that these tests are used for abacavir, thiopurine drugs (TPMT testing) and possibly *CYP3A5* testing for tacrolimus.

A number of tests have been detailed where there is evidence that there is a correlation with the result of the PGX tests with pharmacokinetics, and, in some cases, adverse events. Clearly more research is required for other tests, but it is our opinion that the tests may still be clinically useful on an individual basis, as it allows for tailoring the dose to obtain near-therapeutic drug concentrations, which in some cases, gives some indication of the risk of adverse reactions or efficacy.

In practice, few PGX tests are used clinically. In 2005, Gardiner surveyed Australian and New Zealand laboratories that offered this testing and found very few doing pharmacogenomic testing.[17] A similar low use of TPMT testing was found in Europe.[55] Corkindale et al. looked at reasons why PGX tests are not used in Adelaide and listed a number of factors.[56] Amongst them were lack of having a clinical authority to use for interpretation, lack of peer recognition of the tests, no understanding of the cost implications of the test and difficulty in getting practical information about the tests. In order to address some of these concerns, more informed professionals are needed who both understand the genetic testing as well as the application to the specific drugs. There are some informational websites which list tests by drug name (generic and/or trade name) and facilitate test selection.[57-59] A clinically relevant report is also required and this has been slow in implementation, especially for drug specific reports. Peer acceptance will be slow but may be accelerated if having the test is either required for obtaining the drug through the Pharmaceutical Benefits Scheme (PBS), as is the case for trastuzumab, or obtaining a Medicare Item. (The only test in Australia with a Medicare Item is HLA-B*5701 for abacavir.) With the emphasis of much new drug discovery aimed at new molecular targets, it is likely that both PBS and Medicare funding will increasingly be applicable and encourage the future utilisation of PGX testing.

Conclusion

This article has listed a number of tests where there is a relationship with pharmacokinetic parameters, clinical efficacy or adverse drug reactions and the genetic testing of drug metabolising enzymes or HLA testing. There are very few of these tests however, where a convincing case has been made to test all patients, pre-treatment. Despite this, testing can give useful information to aid in the prescription of certain drugs and drug classes. Education of the public and the medical profession regarding the balanced use of PGX testing is essential to increase their utilisation. PGX testing has the potential to change the way drugs are prescribed and dosage is determined. This is one of the first steps in the pathway to personalised medicine.

Competing Interests: Leslie Sheffield is a director of the pharmacogenetics company GenesFX Health. He intends to report on the clinical interpretation of pharmacogenetics tests in the future as a commercial venture. Hazel Phillimore is an employee at GenesFX Health.

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