Pharmacogenetics in drug regulation: promise, potential and pitfalls

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Pharmacogenetic factors operate at pharmacokinetic as well as pharmacodynamic levels—the two components of the dose–response curve of a drug. Polymorphisms in drug metabolizing enzymes, transporters and/or pharmacological targets of drugs may profoundly influence the dose–response relationship between individuals. For some drugs, although retrospective data from case studies suggests that these polymorphisms are frequently associated with adverse drug reactions or failure of efficacy, the clinical utility of such data remains unproven. There is, therefore, an urgent need for prospective data to determine whether pre-treatment genotyping can improve therapy. Various regulatory guidelines already recommend exploration of the role of genetic factors when investigating a drug for its pharmacokinetics, pharmacodynamics, dose–response relationship and drug interaction potential. Arising from the global heterogeneity in the frequency of variant alleles, regulatory guidelines also require the sponsors to provide additional information, usually pharmacogenetic bridging data, to determine whether data from one ethnic population can be extrapolated to another. At present, sponsors explore pharmacogenetic influences in early clinical pharmacokinetic studies but rarely do they carry the findings forward when designing dose–response studies or pivotal studies. When appropriate, regulatory authorities include genotype-specific recommendations in the prescribing information. Sometimes, this may include the need to adjust a dose in some genotypes under specific circumstances. Detailed references to pharmacogenetics in prescribing information and pharmacogenetically based prescribing in routine therapeutics will require robust prospective data from well-designed studies. With greater integration of pharmacogenetics in drug development, regulatory authorities expect to receive more detailed genetic data. This is likely to complicate the drug evaluation process as well as result in complex prescribing information. Genotype-specific dosing regimens will have to be more precise and marketing strategies more prudent. However, not all variations in drug responses are related to pharmacogenetic polymorphisms. Drug response can be modulated by a number of non-genetic factors, especially co-medications and presence of concurrent diseases. Inappropriate prescribing frequently compounds the complexity introduced by these two important non-genetic factors. Unless prescribers adhere to the prescribing information, much of the benefits of pharmacogenetics will be squandered.

Discovering highly predictive genotype–phenotype associations during drug development and demonstrating their clinical validity and utility in well-designed prospective clinical trials will no doubt better define the role of pharmacogenetics in future clinical practice. In the meantime, prescribing should comply with the information provided while pharmacogenetic research is deservedly supported by all concerned but without unrealistic expectations.

Keywords: adverse drug reactions; dose–response; drug interactions; ethnic differences; pharmacogenetics; regulatory guidelines

1. INTRODUCTION

This paper reviews the implications of genetic influences on dose–response relationship of a drug and the current approaches to integrating pharmacogenetics in drug development. It also summarizes the regulatory framework that supports exploration of pharmacogenetic

influences during drug development and examines future challenges in genotype-driven development and evaluation of new chemical entities (NCE) and their clinical use.

During the clinical use of a drug at present, a prescribing physician has no means of predicting the response of an individual patient to a given drug. Invariably, some patients fail to respond beneficially as expected whereas others experience adverse drug reactions (ADRs). Prescribing of drugs is therefore a relatively empirical trial and error process, frequently resulting in changes in the choice of a drug and/or its dose. Since the majority of ADRs are type A (A for augmentation and result from increased plasma concentrations) pharmacological reactions, they

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ought to be predictable and preventable. This is a highly desirable goal since a number of studies have consistently shown that ADRs and their management constitute a substantial burden on healthcare resources.

These unexpected responses to drugs frequently result from relatively rigid 'standard' dose schedules that are usually recommended, ignoring the diverse interindividual variability within the patient population. Hitherto, drugs have been developed, approved and marketed on a 'one-size-fits-all' basis from population-based mean data on dose, efficacy and safety. The broad assumption is that all patients are a homogeneous group showing little or no interindividual variability. This is evident from drug development programmes that have traditionally tended to minimize or eliminate, rather than embrace, variability in the populations randomized into clinical trials and focused on a very narrow dose range.

Pharmacokinetics and pharmacodynamics are the two key components of the dose–response relationship of a drug—the response being a desired therapeutic effect or an undesirable ADR. Frequently, there are wide interindividual variations in the pharmacokinetics (influencing dose–concentration relationship) or the pharmacodynamics (influencing concentration–response relationship) of a drug. These variations arise from non-genetic as well as genetic influences on the functional activity of drug metabolizing enzymes or pharmacological responsiveness of various drug targets. In terms of genetic influences, the presence of variant alleles often exerts influences that far exceed those due to other covariates that are usually investigated during drug development, for example age, gender, co-medications or the presence of concurrent diseases such as renal or hepatic dysfunction. It is therefore not surprising that the genetic profile (genotype) of an individual may significantly govern the safety and efficacy outcomes (phenotype) following pharmacological interventions clinically. Indeed, genetic factors as a cause of variations in drug responses have long been suspected.

2. PHARMACOGENETIC INFLUENCES ON PHARMACOKINETICS

Much of the attention in pharmacogenetics has hitherto focused on pharmacokinetics. This is not altogether surprising since drug levels are easily measured and correlated to clinical response ('therapeutic drug monitoring'). Furthermore, it was at the level of drug metabolizing enzymes that genetic factors were first found to influence drug response. It is now evident that most drug metabolizing enzymes are expressed in genetically variant forms with altered functional properties.

One of the earliest examples of a genetically determined variation in drug response is the prolonged apnoea that follows administration of suxamethonium chloride (a muscle relaxant) in some individuals. This was found to be due to the inheritance of a variant form of plasma esterase, butyrylcholinesterase (designated atypical cholinesterase). However, it was not until late 1950s that studies on *N*-acetylation of isoniazid, a drug

widely used for the treatment of tuberculosis, resulted in the first systematic characterization of genetic polymorphism in drug metabolizing enzymes (Evans *et al.* 1960; Sunahara *et al.* 1961). A population could be divided into slow or rapid acetylators. Subsequently, variations in responses to a number of drugs metabolized by acetylation were shown to be associated with *N*-acetylation status (slow or rapid acetylators) of individual patients (Evans 1996; Furet *et al.* 2002; Hiratsuka *et al.* 2002).

However, the number of drugs metabolized primarily by acetylation is very few. By far the vast majority of drugs are metabolized by enzymes that belong to a superfamily known as cytochrome P450 or CYPs. Between 50 and 60% of drugs undergoing metabolic elimination are metabolized by CYP enzymes (also known as isoforms). Although a large number of these CYP isoforms are known to occur in nature, the ones that metabolize a vast majority of drugs used clinically are CYP2D6, CYP3A4, CYP2C19 and CYP2C9 (Daly 2004). Other isoforms involved relatively less frequently are CYP1A2, CYP2A6, CYP2B6, CYP2C8 and CYP2E1. Increasingly, genetic polymorphisms are being uncovered not only in these CYP isoforms but also in other drug metabolizing enzymes that are relevant to the development and clinical use of medicines (Daly 2003). These include various methyltransferases (Weinshilboum 1984) including thiopurine *S*-methyltransferase (TPMT) (Schaeffeler *et al.* 2004), UDP-glucuronosyltransferases (UGT) (Burchell 2003; Guillemette 2003), sulfotransferases (Coughtrie *et al.* 1999; Carlini *et al.* 2001) and dihydropyrimidine dehydrogenase (Gardiner *et al.* 2002).

The potential clinical impact of pharmacogenetically determined variability in the activity of drug metabolizing enzymes, and therefore on a drug response, is best illustrated by genetic polymorphism of CYP2D6. It is not only the first CYP polymorphism to be discovered and whose molecular basis was determined but it is also the most widely studied and best characterized. Studies in mid-1970s showed that any given population may be divided into two CYP2D6 drug metabolizing phenotypes—extensive metabolizers (EMs) or poor metabolizers (PMs)—depending on their ability to mediate CYP2D6-dependent hydroxylation of the (now obsolete) antihypertensive drug debrisoquine (Mahgoub *et al.* 1977; Eichelbaum *et al.* 1979; Evans *et al.* 1980). This polymorphism results from autosomal recessive inheritance, in a simple Mendelian fashion, of alleles at a single locus on chromosome 22. Well over 70 CYP2D6 alleles have been identified to date and the details of these can be accessed from a specially dedicated website at <http://www.imm.ki.se/cypalleles/>.

Since the wild type allele (CYP2D6*1) responsible for normal functional activity is dominant, only those individuals carrying two CYP2D6 inactivating alleles (e.g. CYP2D6*3, CYP2D6*4, CYP2D6*5 or CYP2D6*6) are phenotypic PMs. However, among the EM phenotype, there are two subgroups of particular interest at either extreme of the EM population distribution. One subgroup, termed the ultrarapid metabolizers (UMs), is comprised of

individuals with gene amplification and possessing multiple copies of the gene for normal metabolic capacity. UM phenotype may also be associated with inheritance of at least two unique alleles of CYP2D6 (CYP2D6*35 and CYP2D6*41). The other group, termed the intermediate metabolizers (IMs), is comprised of a heterozygous genotype ('gene-dose effect') with a moderate impairment in drug metabolizing capacity.

The pharmacokinetic consequences of polymorphism in CYP2D6, summarized in table 1, are that relative to EMs, the PMs experience far greater exposure to the parent drug (Idle & Smith 1984) and a markedly reduced exposure to the metabolites generated by this enzyme. In contrast, UMs are exposed to high concentrations of rapidly accumulating metabolites and even at very high doses, attain only low plasma levels of the parent drug. Retrospective candidate gene association studies on cases and controls have shown that the PM genotype is associated with an increased risk of a number of ADRs to drugs that are primarily cleared by CYP2D6-mediated metabolism as well as being at risk of lack of efficacy when the therapeutic effect of a drug is mediated principally by its CYP2D6-generated metabolite. For example, since PMs cannot carry out the metabolic activation of the pro-analgesic drug codeine to morphine, they fail to derive adequate analgesic efficacy from codeine. In contrast, UM patients fail to respond to conventional doses of drugs metabolized by CYP2D6 when the therapeutic activity resides in the parent drug and often require 'megadoses' of the drug concerned e.g. the antidepressant nortriptyline or the antianginal drug perhexiline. UM patients are also at risk of toxicity from rapidly accumulating metabolites. For example, rapid conversion of codeine to morphine in these individuals predisposes them to morphine toxicity (e.g. epigastric pain). Table 2 summarizes other important clinical outcomes that may be associated with the above pharmacokinetic consequences of CYP2D6-mediated metabolism.

It is not only the ADRs and efficacy of a drug when used clinically as a single agent that are influenced by CYP2D6 genotype of the patient. Drug-drug interactions also show dramatic inter-genotypic differences. For example, CYP2D6 PMs (with alleles expressing no functional enzyme) do not show the drug-drug interactions predicted from *in vitro* studies. This is hardly surprising since there is no functional CYP2D6 activity that can be inhibited. In contrast to some other drug metabolizing enzymes, CYP2D6 is not inducible (Eichelbaum *et al.* 1986; Wadelius *et al.* 1997; Dilger *et al.* 1999; Branch *et al.* 2000). Likewise, UMs too may fail to exhibit the expected drug-drug interaction. In contrast to PMs and EMs, the UMs have a sufficiently large functional reserve of CYP2D6 activity that they would most probably need much higher (and potentially toxic) doses of the inhibitor to elicit an interaction (Dalen *et al.* 1998; Dalen *et al.* 2000). Under normal conditions of use, the individuals most likely to display a drug interaction are those who have an intermediate or otherwise compromised drug metabolizing capacity (IMs) or those who have inherited CYP2D6 alleles with reduced or altered affinity for CYP2D6 substrates.

Table 1. Pharmacokinetic consequences of CYP2D6 polymorphism.

pharmacokinetic parameter	consequences for the PM relative to EM
bioavailability	2–5 fold
systemic exposure	
C_{max}	2–6 fold
AUC	2–5 fold
half life	2–6 fold
metabolic clearance	0.1–0.5 fold

Table 2. Clinical consequences for PM and ultrarapid EM phenotypes of CYP2D6.

clinical consequences for the PM	
<i>increased risk of toxicity</i>	
debrisoquine	postural hypotension and physical collapse
sparteine	oxytocic effects
perphenazine	extrapyramidal symptoms
flecainide	possibly ventricular tachyarrhythmias
perhexiline	neuropathy and hepatotoxicity
phenformin	lactic acidosis
propafenone	CNS toxicity and bronchoconstriction
metoprolol	loss of cardioselectivity
nortriptyline	hypotension and confusion
terikalant	excessive prolongation in QT interval
dexfenfluramine	nausea, vomiting and headache
L-tryptophan	eosinophilia-myalgia syndrome
indoramin	sedation
thioridazine	excessive prolongation in QT interval
tramadol	hyper-anticoagulation from warfarin
<i>failure to respond</i>	
codeine	poor analgesic efficacy
tramadol	poor analgesic efficacy
opiates	protection from oral opiate dependence
clinical consequences for the ultrarapid EM	
<i>increased risk of toxicity</i>	
encainide	possibly proarrhythmias
codeine	morphine toxicity
<i>failure to respond</i>	
nortriptyline	poor antidepressant efficacy at normal doses
propafenone	poor antiarrhythmic efficacy at normal doses
tropisetron	poor antiemetic efficacy at normal doses
ondansetron	poor antiemetic efficacy at normal doses

At the level of CYP2D6, the anticipated dependence of drug interactions on the metabolic phenotype has already been confirmed for a number of CYP2D6 substrates, for example encainide (Turgeon *et al.* 1990), mexiletine (Turgeon *et al.* 1991), desipramine (Brosen *et al.* 1993), propafenone (Morike & Roden 1994), codeine (Caraco *et al.* 1999) and metoprolol (Hamelin *et al.* 2000). It should be obvious that in PMs, interactions at alternative pathways of metabolism can nonetheless still occur. The point is well

illustrated by an interesting, but expected, genotype-dependent interaction between propafenone and rifampicin—an enzyme inducer (Dilger *et al.* 1999). CYP2D6 that primarily metabolizes propafenone (by 5-hydroxylation) is non-inducible. However, coadministration of rifampicin decreased the oral bioavailability of propafenone from 30 to 10% in EMs and from 81 to 48% in PMs. Following oral propafenone, clearances through *N*-dealkylation (mediated by CYP3A4 and CYP1A2) and glucuronidation, but not CYP2D6-mediated 5-hydroxylation, increased regardless of CYP2D6 phenotype indicating substantial enzyme induction of these enzymes. Thus, induction of non-CYP2D6 pathways by rifampicin resulted in a clinically relevant metabolic drug interaction with propafenone that was more pronounced in EMs than in PMs with regard to percentage decrease in bioavailability of propafenone.

The efficacy of proton pump inhibitors such as omeprazole and lansoprazole, both metabolized by CYP2C19, in reducing gastric acid secretion is due to parent drugs. Studies have shown that PMs of CYP2C19 have higher therapeutic response rates while EMs of CYP2C19 generally require higher doses of these drugs (Klotz *et al.* 2004). Arising from low gastric acidity, subjects of CYP2C19 PM genotype tend to have lower serum levels of vitamin B₁₂ following long-term treatment with omeprazole (Sagar *et al.* 1999) and presumably other proton pump inhibitors. The antimalarial drug proguanil is activated by CYP2C19 to therapeutically more potent cycloproguanil, a strong dihydrofolate reductase inhibitor, and therefore, PMs of CYP2C19 may be at risk of inadequate antimalarial efficacy (Kaneko *et al.* 1999b). The dependence of drug–drug interactions on genotype has also been reported at CYP2C19 (Cho *et al.* 2002; Suzuki *et al.* 2003; Itagaki *et al.* 2004; Wang *et al.* 2004; Yasui-Furukori *et al.* 2004a,b).

Apart from acetylation, polymorphisms of other conjugation reactions such as glucuronidation mediated by UGTs are now also attracting increasing attention, especially in the field of oncology. Glucuronidation is by far the most important conjugation pathway in man. A multigene family encodes the UGTs and a relatively small number of human UGT enzymes catalyse the glucuronidation of a wide range of structurally diverse endogenous (bilirubin, steroid hormones and biliary acids) and exogenous chemicals. Genetic variations and single nucleotide polymorphisms (SNPs) within the UGT genes are remarkably common (Burchell 2003; Guillemette 2003). Two major isoforms of UGT, UGT1A1 and UGT1A9, show wide inter-individual variability in their activities and display genetic polymorphisms that have a significant pharmacological impact in terms of ADRs. Whereas irinotecan and flavopiridol are metabolized by UGT1A, tranilast and atazanavir inhibit this isoform. Retrospective studies investigating the role of UGT1A isoforms in the safety of irinotecan (Ando *et al.* 2000; Iyer *et al.* 2002; Marcuello *et al.* 2004; Rouits *et al.* 2004), flavopiridol (Innocenti *et al.* 2000; Ramirez *et al.* 2002), tranilast (Danoff *et al.* 2004) and atazanavir (Shaw 2002) have been most valuable in explaining the

clinical concerns (myelosuppression, diarrhoea or hyperbilirubinaemia) associated with these drugs.

Long after the removal of troglitazone (a novel and valuable 2,4-thiazolidinedione oral hypoglycaemic agent with insulin-sensitizing activities) from the market due to its frequent, severe and often fatal hepatotoxicity, Watanabe *et al.* (2003) undertook a case–control study of 25 cases of troglitazone-induced hepatotoxicity and 85 controls that investigated 68 polymorphic sites in 51 candidate genes related to a variety of biochemical functions. They reported a strong correlation between elevations of transaminases and the combined glutathione-*S*-transferase GSTT1-GSTM1 null genotype (odds ratio 3.69, 95% CI of 1.354–10.066, $p=0.008$). This retrospective observation not only raises the possibility of a strong genetic substrate in troglitazone-induced hepatotoxicity but also emphasizes the need for candidate gene association studies to investigate associations with a panel of many diverse genes.

3. PHARMACOGENETIC INFLUENCES ON PHARMACODYNAMICS

It has been known for some time now that as with polymorphisms of drug metabolizing enzymes, pharmacological targets of drugs also display genetic polymorphisms and that these too influence drug response. Variant alleles are known to occur not only at the genes expressing target enzymes, channels and receptors but also at the genes responsible for intracellular signal transduction. Better characterized among these pharmacodynamic polymorphisms are the targets involved in cardiac arrhythmias (mutations of sodium and potassium channels), asthma (mutations of β 2-adrenoceptors and of the core promoter of 5-lipoxygenase), cardiac failure (mutations of β 2-adrenoceptors) and depression (mutations of the promoter region of serotonin transporter). While most individuals may respond ‘normally’ as expected, individuals with a genetic variant of a pharmacological target may exhibit a quantitatively or qualitatively different—exaggerated, inadequate or unexpected—response even when the concentration of the drug is within the population-based normal therapeutic range.

The QT interval of the surface electrocardiogram (ECG) reflects the duration of ventricular action potential that is determined by a net balance between inward depolarizing and outward repolarizing currents, especially during phase 3 of the action potential. The major determinant of the outward repolarizing current is known as IKr and is conducted by the rapid component of the delayed rectifier potassium channel. Reduction in this current results in QT interval prolongation. Excessive prolongation of the QT interval often leads to potentially fatal ventricular tachyarrhythmias, particularly a variety known as *torsade de pointes* (TdP). Over the last 10 years, many non-antiarrhythmic drugs have attracted considerable clinical and regulatory interest because of their potential to prolong the QT interval and induce TdP (Shah 2002). These drugs target and inhibit primarily the IKr current.

Following advances in molecular biology, genetics and pharmacology of ion channels, it has become evident that there is a great diversity of genes that control the expression of these potassium channels (Escande 2000). Mutations of the genes that encode subunits of these channels are common and give rise to congenital long QT syndromes (LQTS). However, in view of the low penetrance of many of these mutations, the size of the population with dysfunctional potassium channels is substantially larger than that diagnosed by ECG recording alone. Because of considerable overlap, measurement of the QT interval alone may not permit an accurate molecular diagnosis in families affected by the congenital long QT syndrome. Not all allele carriers have symptoms and only the DNA markers make it possible to reach a genetic diagnosis in these individuals (Vincent *et al.* 1992; Saarinen *et al.* 1998; Priori *et al.* 1999). Although the affected individuals have a normal ECG phenotype, they have diminished repolarization reserve nonetheless and are highly susceptible to drug-induced QT interval prolongation and/or TdP, even at the recommended doses that are normally safe. Individuals who develop drug-induced prolongation of QT interval with or without TdP are not usually genotyped but available evidence suggests that a substantial proportion of the cases of the drug-induced long QT syndrome might represent cases of *forme fruste* of the congenital long QT syndrome. Shah (2004) has recently reviewed the pharmacogenetics of drug-induced QT interval prolongation and TdP.

Regarding polymorphisms of target receptors, individuals who carry Arg16/Gly16 or Gly16/Gly16 variants of β_2 -adrenoceptors have been shown to display a much less favourable immediate bronchodilatory response to salbutamol, in contrast to those with wild type receptor characterized by Arg16/Arg16 genotype. Polymorphisms of β_2 -adrenoceptors may also influence airway responses to regular inhaled β -agonist treatment. Patients with Arg16/Arg16 genotype who use salbutamol regularly show a small decline in morning peak expiratory flow (AM PEF). By the end of a 16-week study, Arg16/Arg16 subjects who had used salbutamol regularly had an AM PEF $30.5 \pm 12.1 \text{ l min}^{-1}$ lower ($p=0.012$) than Arg16/Arg16 patients who had used salbutamol only intermittently as needed. Subjects with Gly16/Gly16 genotype showed no such decline. Evening PEF also declined in the Arg16/Arg16 regular users but not in those who used it intermittently on as-needed basis (Israel *et al.* 2000). Similarly, asthmatic patients who carry mutations of the core promoter of 5-lipoxygenase (ALOX-5) respond poorly to ALOX-5 inhibitors such as zileuton (Drazen *et al.* 1999). Among patients with cardiac failure, those who are homozygous for the Gln27 allele of the β_2 -adrenoceptor display a significantly lower proportion of good responders to treatment with carvedilol than do the patients who are homozygous or heterozygous for the Glu27 allele (26% versus 63%, $p=0.003$) (Kaye *et al.* 2003). Cardiac failure patients who carry a β_2 -adrenoceptor allele with a mutation at codon 164 (Thr164/Ile164) have a 1-year survival of 42% in contrast to 76% in those carrying the normal wild type allele (Thr164/Thr164) (Liggett *et al.* 1998). Provided this observation can be replicated

widely, it would argue for an earlier intervention (including cardiac transplantation) in the former group.

Genetic polymorphism in the promoter region of the serotonin transporter (5-HTT) gene is reportedly a determinant of response to fluvoxamine, a selective serotonin re-uptake inhibitor (SSRI). The insertion variant of this polymorphism (long allele) is associated with higher expression of brain 5-HTT compared to the deletion variant (short allele) (Weizman & Weizman 2000). Patients who have one or two copies of the long variant (homozygous l/l or heterozygous l/s) may show a better therapeutic response than patients who are homozygous for the short variant (s/s). The safety and efficacy of a number of other SSRIs have also been shown to correlate with these 5-HTT genotypes (Kim *et al.* 2000; Pollock *et al.* 2000; Arias *et al.* 2003; Perlis *et al.* 2003; Durham *et al.* 2004).

There are now ever increasing numbers of reports of clinically highly relevant polymorphisms in other pharmacological targets.

4. PHARMACOGENETICS AND REGULATORY FRAMEWORK

Given that genetic factors frequently determine inter-individual and inter-ethnic differences in pharmacokinetics and pharmacodynamics of a drug—with all the associated implications for failure of efficacy in some patients and predisposition to ADRs and drug interactions in others—it is not surprising that regulatory authorities have long recognized the significance of pharmacogenetics in drug development and are now increasingly directing their attention to addressing issues that may arise from genetic heterogeneity of the target patient population.

A number of guidelines from the European Union's Committee for Proprietary Medicinal Products (CPMP), now known as Committee for Medicinal Products for Human Use (CHMP), and the International Conference on Harmonization (ICH) already make direct or indirect references to the need for addressing genetic factors when developing an NCE (table 3).

Indeed, the guideline on 'Pharmacokinetic studies in man', adopted by the CPMP as long ago as February 1987, was probably the first regulatory guideline to include direct references to genetic factors in determining drug response (CPMP 1998). This guideline recommends that metabolic studies should indicate whether the metabolism of a drug may be substantially modified in a case of genetic enzyme deficiency and whether saturation of metabolism may occur, thereby resulting in nonlinear kinetics, within the dose levels normally used.

The ICH guideline on 'Dose-response information to support drug registration' describes how helpful the knowledge of the shape of individual dose-response curves is and it distinguishes individual curves from the population curve (CPMP/ICH 1995). The guideline clearly warns that "Choice of a starting dose might also be affected by potential intersubject variability in pharmacodynamic response to a given blood concentration level, or by anticipated intersubject

Table 3. Pharmacogenetics and CPMP and ICH guidelines.

<i>genetic factors in pharmacokinetics</i>	
1	pharmacokinetic studies in man
2	investigation of drug interactions
3	ICH—ethnic factors in the acceptability of foreign clinical data
4	investigation of bioavailability and bioequivalence
5	ICH—dose–response information to support drug registration ‘...metabolic polymorphism...’
<i>genetic factors in pharmacodynamics</i>	
6	ICH—dose–response information to support drug registration ‘variability in pharmacodynamic response...’

pharmacokinetic differences, such as could arise from nonlinear kinetics, metabolic polymorphisms or a high potential for pharmacokinetic drug–drug interactions” and recommends that in utilizing dose–response information, the influences of various demographic features, individual characteristics (including metabolic differences) and concurrent drugs and diseases should be identified as far as possible.

Since drug interactions are genotype-dependent as discussed earlier, the CPMP guideline on ‘Investigation of drug interactions’ recommends that when performing mechanism-based *in vivo* studies, either when studying the effects of inhibition or induction on the pharmacokinetics of an NCE, consideration should be given to pharmacogenetic factors (CPMP 1997). Subjects participating in metabolic *in vivo* interaction studies should be appropriately genotyped and/or phenotyped (with respect to their drug metabolizing capacity) at the beginning of the study if any of the enzymes mediating the metabolism of the interacting drugs are polymorphically distributed in the population. As an extension to this, although not explicitly stated in the guideline, genotype of the donor liver used for *in vitro* microsomal studies should also be ascertained. This CPMP guideline also recommends investigation of drug interactions at sites other than metabolic route such as renal excretion and transport by efflux pumps and P-glycoprotein. Pharmacogenetic factors are also likely to be important when considering and evaluating drug interactions at these transporters and/or P-glycoprotein.

The CPMP guidance note on ‘Investigation of bioavailability and bioequivalence’ also recommends that phenotyping and/or genotyping of subjects may be considered for safety or pharmacokinetic reasons (CPMP 2001).

The US Food and Drug Administration (FDA) issued in April 1997 their guidance note ‘Drug metabolism/drug interaction studies in the drug development process: studies *in vitro*’ (FDA 1997). This states “Identifying metabolic differences in patient groups based on genetic polymorphisms, or on other readily identifiable factors such as age, race, and gender, could help guide the design of dosimetry studies for such populations groups. This kind of information also will provide improved dosing recommendations in product labelling, facilitating the safe and effective use of a drug by allowing prescribers to anticipate necessary dose adjustments. Indeed, in some cases, understanding how to adjust dose to avoid toxicity may allow the marketing of a drug that would

have an unacceptable level of toxicity were its toxicity unpredictable and unpreventable”. The Japanese Ministry of Health, Labour and Welfare’s drug regulatory authority (Koseisho, now known as the Pharmaceuticals and Medical Devices Agency, PMDA) has also issued guidelines in June 2001 that recommend genotyping in all drug development programmes for drugs that are metabolized by cytochrome P450s (MHLW 2001a,b).

Although the requirements to address these genetic factors are stated in different terms by different regulatory bodies, the net effect of these requirements is that new knowledge concerning pharmacogenetic variations in drug disposition (pharmacokinetics) or responsiveness of pharmacological targets (pharmacodynamics) will lead to additional requirements for pharmacogenetic documentation for NCEs.

5. PHARMACOGENETICS AND GLOBAL DRUG DEVELOPMENT

In today’s age, drug development programmes are undertaken at a global level. This is aimed at reducing the costs, expediting the drug development process and addressing the issues arising from global prescribing of drugs. However, the relative frequency of various alleles of drug metabolizing enzymes varies in different populations. Consequently, the frequencies of the PM phenotype as well of those with intermediate drug metabolizing capacity also show a marked global heterogeneity. For example, the frequency of CYP2D6 PM phenotype is much higher in population groups of western Caucasian origin (5–10%) than in Far East and Asian ethnic groups (0–2%) (Bradford *et al.* 1998; Bradford 2002; Mizutani 2003; Shimizu *et al.* 2003; Ozawa *et al.* 2004). The frequency of PMs of CYP2C19 is lower in western Caucasians (2–4%) compared to the frequencies observed among Orientals (about 15–25%), reaching as high as 60–70% in Vanuatu and other Pacific islands (Kaneko *et al.* 1999c; Xie *et al.* 2001). Global heterogeneity and inter-ethnic differences have also been reported in the frequency of variant alleles of the genes expressing many other drug metabolizing enzymes (Lin *et al.* 1994; Collie-Duguid *et al.* 1999; Hon *et al.* 1999; Scordo *et al.* 2001; Lee *et al.* 2002; Xie *et al.* 2002) and of the gene expressing P-glycoprotein (MDR1, also known as ABCB1) (Ieiri *et al.* 2004; Marzolini *et al.* 2004a). For example, whereas the variant allele styled as MDR1*2 occurred in 62% of European Americans, it was reported in only 13% of African Americans (Kim *et al.* 2001), although the functional or clinical

significance of most of the alleles of MDR1 is controversial and not yet adequately characterized (Chowbay *et al.* 2003; Eichelbaum *et al.* 2004; Soranzo *et al.* 2004).

As with drug metabolizing enzymes, there also exist inter-ethnic differences in the mutations of a number of pharmacological targets. For example, the frequencies of Arg16/Gly16 mutation of β 2-adrenoceptor (also responsible for enhanced agonist-promoted down-regulation and more frequent in nocturnal asthma) in Caucasians, blacks and Asians are 0.61, 0.50 and 0.57, respectively (Weir *et al.* 1998). In terms of haplotypes of mutations of β 2-adrenoceptor, thirteen of the SNPs on β 2-adrenoceptor gene are organized into 12 haplotypes out of the theoretically possible 8192 combinations (Drysdale *et al.* 2000). Four of the observed haplotypes were found in all the four populations sampled (Caucasian, African American, Asian, and Hispanic Latino), although at markedly different frequencies, with one of them showing a more than 20 fold difference in its frequencies. Likewise, while the frequency of long allele of 5-HTT may be as high as 87% in some African American populations, it is as low as 56% in some European Americans (Lotrich *et al.* 2003).

Regulatory guidelines also recognize the potential inter-ethnic differences in pharmacokinetics and pharmacodynamics that might result from this global genetic heterogeneity. Information is therefore required on ethnic demography of clinical trial populations and potential ethnic influences on drug response (CPMP/ICH 1998). In terms of drug development, the significance of these requirements lies in the facts that (i) there is an increasing globalization of drug development with clinical trials often conducted in a geographical population which may not be the ultimate target of the drug, (ii) more and more of the NCEs are found to be substrates of polymorphic drug metabolizing enzymes and/or targeted towards polymorphic receptors—this information is usually not known for older drugs already on the market and (iii) that modern drugs are more potent with narrow therapeutic indices and therefore, relatively small differences in either the pharmacokinetics or pharmacodynamics may become highly relevant clinically. Inter-ethnic differences in drug response are well known (Xie *et al.* 2001) and therefore, the sponsors of NCEs are now anxious to address the issues arising from global prescribing of their drugs. It is also evident that drug–drug interactions too may depend on ethnicity (Caraco *et al.* 1995).

The ICH guideline on 'Ethnic factors in the acceptability of foreign clinical data' recommends the sponsors and the regional regulatory authority in a new region to assess an application for registration for the ability to extrapolate to the new region those parts of the application based on studies from the trial region (CPMP/ICH 1998). To this end, it is recommended that the submission should include (i) adequate characterization of pharmacokinetics, pharmacodynamics, dose–response, efficacy and safety in the population of the trial region and (ii) characterization of pharmacokinetics, pharmacodynamics and dose–response in the new region. The guideline recognizes

the role of genetic factors and when inter-ethnic differences are anticipated, bridging studies may be required. Often, such studies are pharmacogenetic in nature but at times, more extensive data may be required.

An argument is often advanced that interindividual variability far exceeds inter-ethnic variability. This is not in dispute but the argument overlooks the reality that it is not ethnicity but the genotype of the trial population that imposes the hurdle. The individuals of one genotype, for example the PMs of CYP2D6, form one distinct subgroup of regulatory interest regardless of their ethnicity. Ethnicity becomes an important issue only when the trial population is not characterized for its genetic profile, inter-genotype differences in pharmacokinetics or pharmacodynamics are not evaluated and the frequency of the variant alleles is substantially different between the trial and the target populations.

6. PHARMACOGENETICS AND DRUG PRESCRIBING INFORMATION

When pharmacogenetic information from early pharmacokinetic studies is considered clinically relevant, regulatory authorities have always reacted with appropriate labelling recommendations. Four drugs best illustrate the current regulatory approach to incorporating candidate gene-based pharmacogenetic data, gathered from prospective clinical pharmacokinetic studies, in the prescribing information when this information is thought to be potentially relevant to safe and effective prescribing.

Thioridazine is metabolized by CYP2D6 and because of the risk of QT interval prolongation and TdP, it is contraindicated in patients with reduced activity of CYP2D6. Sertindole, an atypical neuroleptic agent, is primarily cleared by CYP2D6. In order to protect the PMs who utilize an alternative pathway mediated by CYP3A4, coadministration of sertindole is contraindicated with ketoconazole and itraconazole, both powerful inhibitors of CYP3A4. The usual dose of the antidepressant escitalopram, the (+)-(S)-enantiomer of racemic citalopram, is 10 mg once daily that may be increased to a maximum of 20 mg daily. However, for patients who are known to be PMs with respect to CYP2C19, the recommendation dose is 5 mg during the first two weeks of treatment which may be increased to 10 mg. Celecoxib, a COX-2 selective inhibitor, is predominantly metabolized by CYP2C9 and, therefore, caution is recommended when celecoxib is prescribed to patients known to be PMs of CYP2C9. Since fluconazole inhibits CYP2C9, it is also recommended that celecoxib should be used at half the normal doses in patients receiving fluconazole. Arising from the wide inter-ethnic differences in the pharmacokinetics of this drug, an initial lower dose is recommended in black patients.

When additional data are or become available, a number of sections of the prescribing information (e.g. dose schedules, contraindications, special warnings and precautions for use, drug interactions, ADRs) may have to be written in terms of pharmacogenetic profile of the patients. The most recently approved drug that best illustrates this expected complexity of

prescribing information is atomoxetine (FDA 2002). This drug, approved by the US FDA in December 2002, is indicated for attention deficit hyperactivity disorder and is metabolized primarily through CYP2D6 and extensive genotype-related information is included in a number of sections of the US labelling including drug interactions and ADRs.

One risk that needs to be highlighted is that unless the positive and negative predictive values of genotype–phenotype associations are high, dosing recommendations based exclusively on genotype may well lead to (sub-therapeutic) under-dosing in some individuals simply because of their genotypes and not necessarily because they are at risk of toxicity. For diseases that are life threatening, this may have serious consequences for the patients concerned. A collective analysis of the data on irinotecan, indicated for metastatic colorectal cancer, well illustrates the point. UGT1A1*28 genotype has a positive predictive value of only 50% and a negative predictive value of 90–95% for toxicity and, therefore, the expert view from the US Clinical Pharmacology Subcommittee in November 2004 was that this test should not be used in isolation but coupled with other information such as monitoring the patient, using a lower dose, pre-existing risk factors (FDA 2004).

7. PHARMACOGENETICS IN DRUG DEVELOPMENT TO DATE

In order to comply with various regulatory recommendations, sponsors of an NCE already conduct formal early pharmacology studies in a genotyped panel of healthy volunteers to characterize pharmacogenetic influences on pharmacokinetics of the NCE. Unfortunately, however, the findings are rarely carried forward to improving the designs and inclusion criteria for subsequent dose finding and pivotal studies.

It is most unusual to see dose finding studies that include information on the genotype of the individuals randomized. It is therefore uncertain that the entire range of variability in dose–response, and dose requirements likely to be encountered in the target population at large, has been studied. This deficiency has serious implications for selecting the most appropriate dose(s) for a pharmacogenetically heterogeneous population that will be randomized into the pivotal studies and, ultimately, for the wider clinical use of the drug. It may be noted that debrisoquine, the prototype substrate of CYP2D6, was found during its post-marketing period to be clinically effective in the dose range extending from 20 to 360 mg daily. In the context of CYP2D6-mediated metabolism, the dose requirements for nortriptyline and perhexiline also illustrate the point (Meyer 2000; Sallustio *et al.* 2002). For therapeutic effect of nortriptyline, UMs require more than 500 mg daily in contrast to PMs who need only 20–50 mg daily (Meyer 2000). There is a range of doses in the groups between these two extremes. Perhexiline, an effective antianginal drug, was associated with disabling neuropathy, hepatitis and weight loss. It was therefore withdrawn from clinical use in 1985. When prescribed at the recommended dose of 100 mg three times a day, PMs are at a much greater

risk of perhexiline-induced neuropathy and hepatitis (Shah *et al.* 1982; Morgan *et al.* 1984). It is now known that to maintain the plasma concentrations of perhexiline within the therapeutic and non-toxic range, PMs require a dose of 10–25 mg daily while EM and ultrarapid EM require 100–250 and 300–500 mg daily, respectively (Sallustio *et al.* 2002). The perils of prescribing a ‘standard dose’ of CYP2D6 substrate drugs to all patients, regardless of their CYP2D6 metabolic capacity, are obvious. This ‘one-size-fits-all’ approach exposes some individuals to concentration-dependent ADRs. Kirchheiner *et al.* (2001) have proposed a preliminary guidance for a number of drugs metabolized by CYP2D6 and CYP2C19 with a view to pioneering genotype/phenotype-specific dose schedules.

Patients in pivotal clinical studies are seldom, if ever, genotyped. Even those patients that withdraw from the studies because of failure of efficacy or development of a serious ADR do not attract any further attention. And yet, these are the patients who are most likely to represent or include outliers of pharmacogenetic interest.

8. LIMITATIONS OF CURRENT FOCUS ON PHARMACOKINETICS

Data from retrospective studies aimed at associating genetically determined variations in drug metabolizing activity (genotype) with variations in drug response (phenotype) raised expectations in the 1980s and 1990s that polymorphisms in drug metabolizing enzymes might substantially explain the lack of efficacy or induction of ADRs associated with their substrate drugs at a population level. If confirmed prospectively, pre-treatment genotyping of patients may offer exciting prospects of removing guesswork from prescribing and improving therapeutic skills. However, despite the variability in pharmacokinetics of a wide range of drugs metabolized by highly polymorphic enzymes such as CYP2D6, there is insufficient evidence to support the notion that these polymorphisms are actually associated with altered outcomes and/or drug toxicity in routine clinical practice (Wedlund & de Leon 2004). Meta-analysis of small-scale studies has shown genotyping to be less promising than had been anticipated. Predictive CYP2D6 genotyping is estimated to be beneficial for treatment of about 30–40% of CYP2D6 drug substrates, that is, for about 7–10% of all drugs used clinically, although prospective clinical studies are necessary to evaluate the exact benefit of drug selection and dosage based on the CYP2D6 genotype (Ingelman-Sundberg 2005). Other drug metabolizing polymorphisms also suffer from similar lack of prospective data on pre-treatment genotyping as a tool to guide prescribing.

The disappointing and often conflicting or ambiguous reports on the clinical significance of genetically determined pharmacokinetic variability may simply indicate the limitations of single candidate gene approach. But there are other more compelling reasons for guarded scepticism regarding the clinical significance of pharmacogenetic variability in pharmacokinetics. In the context of polymorphic drug

metabolizing enzymes, some limitations in applying pharmacogenetics to therapeutics are already self-evident. These are:

- (i) Pharmacokinetic variability will likely be relevant only when the pharmacology (activity and potency) of the parent drug and its metabolite is significantly different and the drug response has a steep concentration–response curve. For those drugs where both the parent drug and the metabolite(s) are pharmacologically active, the consequences of defective metabolism would depend on the overall contribution of the parent drug and the metabolite(s) to the therapeutic (or toxic) effects of the drug.
- (ii) Very few drugs are metabolized by a single enzyme. Furthermore, PMs are often able to utilize alternative, but often less effective, pathways of elimination.
- (iii) Many drug metabolizing enzymes are subject to variant alleles which express enzymes with altered substrate specificity or altered functional activity.
- (iv) Not all ADRs of a drug are genetically determined even if a drug is metabolized by a single pathway. Whereas only one or two of these may have genetic basis, others that are often responsible for limiting the treatment have no obvious genetic basis. Small systematic studies suggest that often, not even the serious ADRs are associated with a specific genotype (Clark *et al.* 2004).
- (v) In addition to drug metabolizing enzymes, P-glycoprotein and associated organic ion transporters also influence the disposition of many drugs. These play an important role in the absorption of drugs and their transport into the cells and elimination into the bile or urine. The activities of these P-glycoprotein and transporters are also polymorphically expressed and genetically determined. Although their pharmacokinetic effects may be moderate, they nevertheless distort associations with other genotypes.
- (vi) Not all toxic effects need have a pharmacokinetic basis.

Available data illustrate an important point from the regulatory perspective. Promises of potential clinical benefits from integrating pharmacogenetics in clinical medicine are often based on inappropriate presumptions on the role of polymorphic drug metabolizing enzymes or pharmacological targets. As discussed below, a number of drugs illustrate this point and often, the results from pharmacogenetic studies of specific drug-induced toxic effects are inconsistent or contradictory.

Thioridazine has been shown in healthy volunteers to have a dose-related effect on ventricular repolarization, primarily due to the parent drug with a possible contribution from the metabolites (Hartigan-Go *et al.* 1996). One recent study reported that CYP2D6 status might be an important determinant of the risk for thioridazine-induced QTc interval prolongation

(Llerena *et al.* 2002) while another reported that CYP2D6 genotype does not substantially affect the risk of thioridazine-induced QTc interval prolongation (Thanaccody *et al.* 2003). This discrepancy is not altogether too surprising since the metabolite probably contributes significantly, but variably between individuals, to this toxic effect.

The selective norepinephrine reuptake inhibitor atomoxetine is metabolized by CYP2D6. The peak plasma concentration ($C_{\max}$) of and the systemic exposure (area under plasma concentration versus time curve, AUC) to the pharmacologically active parent drug are five and 10 fold, respectively, higher in PMs compared to the EMs. However, when the safety profile of this drug is scrutinized in terms of CYP2D6 genotype (e.g. percentage of patients of each genotype discontinuing therapy because of a side effect), it is questionable if pre-treatment genotyping of patients is cost-effective or of any value clinically given the positive and negative predictive values of the association. Atomoxetine is metabolized by dual pathways—predominantly by CYP2D6 to pharmacologically equipotent 4-hydroxy-atomoxetine and to a lesser extent by CYP2C19 to almost inactive *N*-desmethyl-atomoxetine. Relative to atomoxetine, the plasma concentrations of 4-hydroxy-atomoxetine and *N*-desmethyl-atomoxetine are about 1 and 5%, respectively, in EMs and 0.1 and 45%, respectively, in PMs. In prospective clinical trials, many neuropsychiatric adverse events were generally only twice as frequent in PMs compared with the EMs and 5% of the EMs and 7% of the PMs discontinued treatment as a result. In terms of absolute numbers, these translate into about seven EMs and one PM for every 100 un-genotyped patients discontinuing treatment (FDA 2002). To further add to this disappointment, the current safety concerns regarding atomoxetine are focussed on its hepatotoxic potential, a safety signal not evident during clinical trials.

Two classes of drugs, antidepressants and neuroleptics, have narrow therapeutic index and are generally metabolized predominantly by CYP2D6. Although small retrospective studies appear to show a correlation between genotype and toxicity or failure to respond (Rau *et al.* 2004), overall analyses of studies correlating CYP2D6 genotype with response to these drugs (referred to as phenotype) have been cautious in their conclusions (Dahl 2002; Kirchheiner *et al.* 2003a, 2004b,c). The author of this paper analysed 17 studies published between 1995 and 2000, which had included over 1350 patients receiving a range of neuroleptic drugs. These studies investigated associations between CYP2D6 genotype and drug levels, failure to respond beneficially, and frequency and severity of a number of ADRs such as neuroleptic malignant syndrome, extrapyramidal symptoms (EPS) and tardive dyskinesia (TD). Relationship with plasma concentrations was shown for drugs with dominant CYP2D6-mediated metabolism but large intra-genotypic variability tended to obscure its clinical value. However, there was no relationship evident between genotype and failure to respond beneficially. There was only a general modest trend observed towards a positive correlation between the genotype, especially the presence of CYP2D6*10 allele in the Japanese, and severity of TD and EPS.

These disappointing findings are hardly surprising since a number of these drugs are also metabolized by pathways other than those mediated by CYP2D6 and frequently, these drugs have metabolites that are pharmacologically active in terms of efficacy and ADRs.

Non-steroidal anti-inflammatory drugs (NSAIDs) are used widely and are responsible for treatment limiting gastro-intestinal side effects or hepatotoxicity. Indeed, hepatotoxicity is the most frequent cause of removal of NSAIDs from the market. Although most NSAIDs are metabolized by CYP2C9, no association between CYP2C9 genotype has been shown for either the gastro-intestinal complications of NSAIDs generally (Martin *et al.* 2001) or the hepatic complications of diclofenac (Aithal *et al.* 2000). A more recent study has also found no evidence of impaired metabolism of oral diclofenac in heterozygous and homozygous carriers of the CYP2C9 alleles *2 and *3 compared with the wild type allele and marked diclofenac-mediated inhibition of COX-1 and COX-2 activity was detected in all individuals independent of CYP2C9 genotype (Kirchheiner *et al.* 2003c). The current consensus is that CYP2C9 genotyping is unlikely to become routine clinical practice unless its value can be demonstrated in rigorous prospective studies (Kirchheiner & Brockmoller 2005).

Azathioprine and 6-mercaptopurine are metabolized by the polymorphic TPMT. The activity of TPMT is inversely related to the risk of developing acute leucopenia. A number of studies have shown that the risk of azathioprine-induced acute leucopenia can be greatly reduced by selecting the initial azathioprine dose based on TPMT genotype or phenotype (Colombel *et al.* 2000; Regueiro & Mardini 2002). However, an analysis of six clinical studies correlating the adverse effects of these drugs with TPMT genotype revealed that an average of 78% of ADRs were not associated with TPMT polymorphism. Pharmacogenetic testing will thus not eliminate the need for careful clinical monitoring of ADRs (Schwab *et al.* 2002; Gearry *et al.* 2003; van Aken *et al.* 2003). Of course, this is not to suggest that it is not worth screening patients for genetic variants that may explain other well-established variations in drug responses but the above analysis does illustrate the limitations of pharmacogenetics in routine clinical practice.

Similarly, CYP2D6 polymorphism has proved disappointing in predicting response to antihypertensive drugs (Kirchheiner *et al.* 2004a; Schwartz & Turner 2004). Drug interactions too are similarly rendered irrelevant if metabolites are pharmacologically active. For example, venlafaxine (a dual mechanism-based antidepressant) is metabolized by CYP2D6 but the labelling of this drug notes that in a clinical study involving CYP2D6 PMs and EMs, the total concentration of active compounds (venlafaxine plus its major metabolite, *O*-desmethylvenlafaxine), was similar in the two genotypes. Therefore, no dosage adjustment is required when venlafaxine is coadministered with a CYP2D6 inhibitor.

Retrospective studies have also suggested that PMs of CYP2C9 are more susceptible to toxicity from warfarin or phenytoin—both drugs with a steep

concentration–response curve—and that there are wide inter-genotypic differences in dose requirements (Kidd *et al.* 2001; van der Weide *et al.* 2001; Higashi *et al.* 2002). Interestingly, however, although CYP2C9 is intricately involved in the elimination of pharmacologically active (*S*)-isomer of warfarin, its role in long-term safety of this widely used anticoagulant has yet to be shown conclusively (Takahashi *et al.* 2003; Kamali *et al.* 2004; Takahashi *et al.* 2004). Available data indicate that although CYP2C9*3/CYP2C9*3 genotype is associated with dramatic over anticoagulation soon after the introduction of this anticoagulant, overdose during the maintenance period is mostly related to environmental factors (Verstuyft *et al.* 2003; Peyvandi *et al.* 2004) which greatly influence inter-individual variability in warfarin sensitivity. In one study, age and CYP2C9 genotype accounted for 12 and 10% of the variation in warfarin dose requirements, respectively (Loebstein *et al.* 2001). Clearly, other factors such as variations in the activity of vitamin K epoxide reductase (VKOR) and diet also play an important role. VKOR is the target of warfarin and there are reports of familial occurrence of defects in a protein of the VKOR-multienzyme-complex (Oldenburg *et al.* 2000). Genetic control in the activity of VKOR has been described (Rost *et al.* 2004) and Li *et al.* (2004) have recently identified the gene for VKOR. Polymorphisms of this VKOR gene may turn out to have a much greater effect on the response to warfarin than its CYP2C9-mediated metabolism. Similarly, despite the role of CYP2C19 in activating the antimalarial pro-drug proguanil to its therapeutically potent metabolite (cycloproguanil), clinical observations do not support the anticipated notion that the drug will be ineffective in PMs of CYP2C19 (Kaneko *et al.* 1999a).

One of the uncertain aspects of pharmacokinetic variability is the extent to which the disposition of many drugs is influenced not only by the drug metabolizing enzymes but also by P-glycoprotein. These are part of a larger family of efflux transporters. They appear to have developed as a mechanism to protect the body from harmful substances. P-glycoprotein is a 170 kDa membrane-bound protein that functions as a membrane-localized drug transport mechanism with an ability to actively pump out a number of drugs. These transporters have been identified at a number of interfaces that the drugs might cross, for example the intestinal wall, choroid plexus, gonads, placenta, renal tubules and biliary canaliculi. Using ATP as an energy source, they transport certain hydrophobic substances into the gut, bile or urine and out of the brain, gonads or other vital organs. Thus, they decrease oral bioavailability of drugs but once absorbed, decrease transfer of drugs across the blood–brain barrier and reduce their concentrations in the central nervous system or across the placenta to the foetus. In liver and kidney, these transporters are actively involved in secretion of drugs into the bile and urine, respectively. The expression of P-glycoprotein activity is under the control of MDR1 (also known as ABCB1) gene (Hoffmeyer *et al.* 2000) and is an important factor in the disposition of many drugs. The processes involved show considerable

interindividual variability that is genetically determined. Allelic variation in MDR1 gene is more common than had been previously recognized and involves multiple SNPs whose allelic frequencies vary between populations, and some of these SNPs are associated with altered P-glycoprotein function. Mutations at positions 2677 and 3435 are associated with alteration of P-glycoprotein expression and/or function. Two synonymous SNPs (C1236T in exon 12 and C3435T in exon 26) and a non-synonymous SNP (G2677T, Ala893Ser in exon 21) are frequently linked and the allele with this haplotype is styled MDR1*2. The AUC of fexofenadine was found to be almost 40% greater in individuals with *1/*1 genotype compared to those with *2/*2 genotype. Those with the *1/*2 heterozygous genotype had an intermediate value. This suggests an enhanced *in vivo* P-glycoprotein activity among subjects with the MDR1*2 allele (Kim *et al.* 2001). This and related variant alleles also exert an influence on the bioavailability and disposition of other drugs, although some data are contradictory (Hoffmeyer *et al.* 2000; Parker *et al.* 2003; Eichelbaum *et al.* 2004; Ieiri *et al.* 2004; Marzolini *et al.* 2004a). Chowbay *et al.* (2003) were able to show clinically relevant substantial differences in the pharmacokinetics of cyclosporin in relation to MDR1 haplotypes (SNPs in exon 12, 21, and 26) in stable heart transplant patients. From the overall evidence available to date, it appears that MDR1 polymorphisms have only a moderate impact on pharmacokinetics and pharmacodynamics of P-glycoprotein substrates (Eichelbaum *et al.* 2004) and it remains unclear what polymorphism may be responsible for which, if any, effects (Soranzo *et al.* 2004).

Organ-specific organic anion and cation transporters are now recognized to play an important role in the transport of some drugs into the cells and their elimination into the bile or urine (Kim 2004; Lee & Kim 2004; Marzolini *et al.* 2004b; Mikkaichi *et al.* 2004). Molecular studies have found evidence of genetic polymorphisms of these transporters in hepatocytes (Zhang *et al.* 1997; Tirona *et al.* 2001; Tirona & Kim 2002; Kim 2004). Mutations in the genes coding for these transporters may lead to dysfunctional polypeptides, which not only affect the pharmacokinetics of the drugs concerned but may also intensify the potential of some of these drugs to induce hepatotoxicity (Murata *et al.* 1998; Fouassier *et al.* 2002).

9. RELATIVE IMPORTANCE OF PHARMACOKINETIC AND PHARMACODYNAMIC POLYMORPHISMS

It is now becoming increasingly evident that polymorphisms of pharmacological targets (pharmacodynamic polymorphisms) may in fact be more important and clinically relevant than polymorphisms of drug disposition (pharmacokinetic polymorphisms). In a pharmacogenetic study that compared paroxetine and mirtazapine in 246 elderly patients with major depression, discontinuations due to paroxetine-induced side effects were strongly associated with the 5-HTR_{2A} C/C, rather than CYP2D6, genotype. There was a significant linear relationship between the

number of C alleles and the probability of treatment discontinuation. The severity of side effect in paroxetine-treated patients with the C/C genotype was also greater (Murphy *et al.* 2003). Thus, although paroxetine is metabolized by CYP2D6, polymorphism of 5-HTR_{2A} appears to be a more important determinant of paroxetine-induced ADRs. In another study of 270 cancer patients given anti-emetic therapy with 5-HTR_{3B} receptor antagonists, approximately 30% suffered from nausea or vomiting despite these drugs. Ultrarapid metabolism of tropisetron (and to a lesser extent for ondansetron) was shown to predispose patients to poor efficacy (Kaiser *et al.* 2002). However, another study by the same group of investigators reported that patients homozygous for a deletion variant of the promotor region of 5-HTR_{3B} experienced vomiting more frequently than did all the other patients. In terms of numbers needed to screen to identify each case of vomiting, 5-HTR_{3B} polymorphism appeared to be complementary to CYP2D6 polymorphism (30 for the combination versus 50 for CYP2D6 alone) (Tremblay *et al.* 2003).

The potentially greater importance of VKOR polymorphism relative to CYP2C9 polymorphism in determining anticoagulant response to warfarin has already been referred to earlier.

Hypolipidaemic responses to HMG-CoA reductase inhibitors vary widely in the population. The systemic exposure to both enantiomers of fluvastatin, an HMG-CoA reductase inhibitor, depends on CYP2C9 genotype, with a three fold group mean difference in the active enantiomer, and even greater difference in the inactive enantiomer, between the EMs and the PMs of CYP2C9. However, these differences in plasma concentrations of fluvastatin were not reflected in the effect on cholesterol levels after 14 days of fluvastatin intake in healthy volunteers (Kirchheiner *et al.* 2003b). Although several human cytochrome P450 enzymes metabolize fluvastatin, the pharmacokinetic differences are not too surprising because CYP2C9 contributes 50–80%. Lack of a difference in pharmacodynamic responses is, however, surprising. In contrast to the apparent clinical irrelevance of CYP2C9 polymorphism in fluvastatin-induced changes in serum cholesterol levels, Chasman *et al.* (2004) have identified two tightly linked SNPs in the gene coding HMG-CoA reductase that are associated with reduced efficacy of pravastatin therapy. It is not unreasonable to expect that this polymorphism will also influence fluvastatin-induced changes in serum cholesterol levels. Compared with individuals homozygous for the major allele of one of the SNPs, individuals with a single copy of the minor allele had 22 and 19% smaller reductions in total cholesterol and LDL-cholesterol, respectively. In retrospect, it is tempting to think that poor efficacy in a few individuals with anomalous target genotype may be the stimulus to driving upwards the recommended doses of HMG-CoA reductase inhibitors in the post-marketing period. For example, the recommended dose of cerivastatin was progressively increased from a maximum of 0.3 mg daily at the time of its approval in 1997 to a maximum of 0.8 mg daily at the time of its withdrawal from the market in August 2001. However, it is uncertain whether the individuals with anomalous

target genotype respond any better to higher doses. Undoubtedly, this must adversely affect the risk/benefit of the drug in the wider population.

Not surprisingly, the focus of current interest in pharmacogenetics of drug response has switched from drug metabolizing enzymes to the candidate genes of the pharmacological targets. Investigation of pharmacodynamic polymorphisms is now an active area of research.

From a regulatory or clinical perspective, it is worth emphasizing that the consequences of polymorphisms in pharmacokinetics may be manageable by adjustment of the dose to achieve the required therapeutic concentrations (provided the toxicity from parent drug or the metabolites does not supervene). However, the consequences of polymorphisms in pharmacological targets are far less likely to be easily managed. Indeed, these polymorphisms may have the effect of subdividing the target population or the disease into discrete subgroups—each requiring drugs that act at different pharmacological targets. This has indeed been the experience with long QT syndrome which was at one time thought to be a single disease but now is recognized to be a heterogeneous group of syndromes with each syndrome having its own natural history and antiarrhythmic response to β -adrenoceptor blocking therapy.

10. PHARMACOGENETICS AND FUTURE DRUG DEVELOPMENT

It is evident from the above discussion that for pharmacogenetics to be a truly valuable drug developing and prescribing tool, pharmacogenetic approaches during drug development will have to be more 'holistic'. These approaches will have to focus on investigating genetic influences not only at pharmacokinetic (drug metabolizing enzymes and transporters) but also at pharmacodynamic (pharmacological targets) levels to fully characterize the pharmacology of drugs. Clearly, a patient's overall genotype that determines a drug response (phenotype) must take into account the presence of normal wild type and mutant alleles in heterozygous and homozygous states at both these key components of the dose-response relationship. The situation becomes even more complex when one also considers the presence of multiple alleles at a single locus.

There are considerable commercial, regulatory and clinical implications for the observation that a particular genetic trait (single candidate gene or a SNP profile) confers susceptibility to toxicity or poor therapeutic response. For example, consider the fate of an NCE that is less effective than an approved drug in the wider un-genotyped population but is found to have a superior efficacy or safety profile in a genotypic subset of the target population. It is therefore not surprising that, following the completion of the Human Genome Project, immense efforts are under way at characterizing 'normal' nucleotide sequences as well as abbreviated profiles of nucleotide polymorphism(s) associated with diseases and with therapeutic responses to drugs. The outcomes from these efforts are anticipated to provide a better and much greater

understanding than has hitherto been possible of genetic factors underlying disease processes, development of new drug targets and biomarkers and responses to drugs. Expectations have been raised, now higher than ever, that the goal of individually targeted therapy can be achieved.

(a) *Establishing genotypelphenotype associations*

Two approaches have been used—candidate gene association studies or genome-wide scans looking for SNP or haplotype profiles associated with drug response. Despite the alleged limitations of candidate gene approach, almost all the pharmacogenetic studies to date have focused on this approach and most successes so far have resulted from it. Candidate gene association is mechanistic and relatively less resource intensive but allows only a few genes to be studied. In contrast, establishing a SNP profile from genome-scan is empirical, requires no knowledge of pharmacology of the drug and allows a much wider search. Therefore, although genome-wide association studies are complex, they may probably be the only way to better characterize the genotype/phenotype relationships. However, it may be too optimistic to believe that all relevant pharmacogenetic variations will be SNPs, especially as we already know examples of large deletions, amplifications and re-arrangements (Idle *et al.* 2000). Genome-wide scans for SNPs may also be limited in their applications since it is known that even at a single candidate gene locus, there is considerable allelic heterogeneity and there are wide inter-ethnic variations in the frequency of various SNPs (Cargill *et al.* 1999; Goddard *et al.* 2000; Stephens *et al.* 2001; Ng *et al.* 2004). The prevailing 'SNP fever' may have to be further tempered in the knowledge that there are virtually no examples where a single DNA variant site (genotype) can always be associated with a particular trait (phenotype) in all subjects within all human populations (Nebert 2000). Nebert *et al.* (2003) have summarized some of the major problems.

Both within and outside the industry, the efforts at fulfilling the expectations of individually targeted therapy are based on two approaches to clinical trials.

(b) *Classical clinical trials integrating pharmacogenetics*

It appears likely that it may soon become possible to routinely and rapidly genotype individuals for a variety of genetic traits at a very nominal cost. In order to explore the role of pharmacogenetics in drug response, sponsors are now including a genetic extension to the usual clinical trial protocols, enabling them to collect and store blood samples for genetic analysis. Although the patients would be required to give informed consent for the main study protocol, the consent to genetic extension will be optional without prejudicing their chance of enrolment in the main study.

The blood samples of those patients who consent would be analysed at a later date for an analysis of genotype/phenotype relationship to establish either a candidate gene or a SNP profile (from genome-wide scan) associated with toxicity or failure to respond. This approach has the advantage of permitting analysis

Table 4. Advantages and disadvantages of enrichment design pharmacogenetic studies.

<i>advantages</i>	
1	reduction in number of dropouts from the study
2	trials with smaller number of patients
3	trials of shorter duration
4	reduction in requirements for safety monitoring
5	exploration of doses higher than otherwise possible
6	elimination or reduction of inter-individual variability
<i>disadvantages</i>	
1	inadequate information regarding potential variability in the target population
2	overestimation of the dose of a drug and its efficacy
3	further erosion of short-term and long-term safety data
4	distorted comparisons in active controlled trials
5	arbitrary exclusion criteria since multiple enzymes frequently involved in drug metabolism
6	disregard for the presence of multiple variant alleles at a given locus, which may have different substrate specificity—which genotypes are candidates for exclusion?
7	not possible to investigate safety and efficacy of even the lower doses in genotypes excluded as proposed and currently applied, this design overlooks the importance of pharmaco-
8	dynamic polymorphisms and of haplotypes

of the samples for specific genotypes related to drug disposition as well as pharmacological targets.

(c) *Enrichment design clinical trials*

Enrichment design studies are another approach favoured by some sponsors. This approach uses pre-enrolment genotyping to exclude (at present focusing on certain genotypes of drug metabolizing enzymes) from randomization those individuals who are unlikely to benefit or are likely to develop ADRs (Murphy 2000; Murphy *et al.* 2000). Not unexpectedly, this design is advocated in order to generate more robust evidence of efficacy but with trials of shorter duration and fewer patients. It is also claimed to increase subject safety and eliminate the need for monitoring drug plasma concentrations. The advocates of this design clearly presume (but seemingly without prospective evidence) a causal role for the genetic trait in drug response.

The current efficacy-orientated approach to clinical trials already results in insufficient characterization of the clinical safety of an NCE. When a pharmacogenetic trait is found to improve efficacy, it is inevitable that clinical trial populations will be highly select and even smaller. The concern then will be further erosion in characterization of the safety of the drug. Regulatory authorities are likely to approach with great caution any clinical development programme heavily dominated by enrichment design studies. The advantages and disadvantages of this design are summarized in table 4.

Clinical development programmes will then be required to undertake specific additional safety and efficacy studies in genotypes excluded from pivotal studies. Since it is very rare for a drug to be withdrawn from the market for failure of efficacy, there exists a more compelling case for conducting very carefully monitored studies in genotypes suspected to be at risk. Thus, prospective genotyping should be used to ensure inclusion of important patient subgroups. The perils of excluding important subgroups from clinical trials are already evident in the withdrawal of numerous drugs that produce TdP or hepatotoxicity—the two serious ADRs that are most frequent in female gender (Makkar *et al.* 1993; Shah 1999)

Probably the most important concern when integrating pharmacogenetics in drug development is the fact that rare or delayed ADRs (which are usually the serious ones and responsible for drug withdrawals) are unlikely to be observed during clinical trials. Perhexiline-induced neuropathy, for example, was not evident during clinical trials, and during its marketing patients with neuropathy had taken the drug for a mean of 20 months. Therefore, in order to truly harness the potential benefits, pharmacogenetic studies will have to continue well beyond the approval of a drug into its post-marketing surveillance period.

Data protection, privacy and sample destruction will be essential components of the consent for these pharmacogenetic protocols (CIOMS 2005). In response to these developments, the CHMP has recently adopted a 'Position paper on terminology in pharmacogenetics' (CPMP 2002). This position paper describes five categories of coding of the blood samples (identified, single-coded, double-coded, anonymized and anonymous) for maintaining patient privacy without compromising the scientific and regulatory objectives of the study.

11. PHARMACOGENETICS AND FUTURE REGULATORY APPROACHES

In future, substantial pharmacogenetic data will be submitted with the dossiers of NCEs, raising issues that will be important for regulatory integration of these data in the overall drug evaluation and approval processes (CIOMS 2005). Whereas extensive pharmacokinetic investigations during clinical development may reveal the efficacy and safety implications of polymorphic pharmacokinetics of a drug, at least one concentration-controlled clinical trial may have to be considered in order to characterize variability in pharmacodynamics. Regulatory aspects most likely to be influenced are assessment of efficacy, dose schedules, ADRs and drug interactions in relation to genotype and communicating this assessment to the prescribing community. Sponsors will seek guidance on how pharmacogenetic data ought to be presented and analysed and may form part of the labelling. Regulators

need to start addressing these issues and articulate specific guidance.

To this end, both the CHMP and the FDA have already implemented measures for 'briefing meetings' or 'voluntary submission of genomic data', respectively (CPMP 2003; FDA 2003). The PMDA in Japan recently issued a public consultation document requesting comments on their proposal for preparation of guideline for the use of pharmacogenomics in clinical trials. The outcome of this consultation has resulted in March 2005 in a final guidance note that is very similar to the FDA guidance on 'voluntary submission of genomic data'. Sponsors of NCEs who wish to explore pharmacogenetics during drug development are encouraged to collect these data and submit these exploratory and preliminary (probably non-validated) data to expert regulatory groups for discussion without prejudice or any fears and concerns about adverse impact from sharing these data on their development programme ('safe harbour'). This concept of 'safe harbour' implies that the exploratory data disclosed to the regulatory authority will be beyond the regulatory 'reach' for initiating any pre-emptive regulatory action. These meetings are intended to provide an informal forum for discussions between the sponsors and the regulators with a view to developing regulatory and scientific understanding and future policies on safety and efficacy paradigms. If requested, the regulators would offer advice on improving or designing studies aimed at generating answers to the questions that may emerge during the regulatory assessment of the data submitted to support an application for a marketing authorization. Such bilateral discussions and examination of pharmacogenetic data from a wide range of drug development programmes should facilitate logical applications of pharmacogenetics to subsequent drug development and, ultimately, to clinical therapeutics.

Regulatory agencies will want to see pharmacogenetic data that are consistently reproducible and more predictive of drug response on an individual drug-by-drug basis before embracing pharmacogenetics in evaluation, approval and labelling of drugs. Indeed, the entire dataset will need careful scrutiny before pre-prescription genotyping can be advocated for any particular drug. In principle, evaluation of data in relation to genotype may appear to be a relatively straightforward procedure comprising assessment of efficacy in clinical trial populations sub-grouped by genotype. In this context, genotype may be regarded as just one more of the many variables that regulatory agencies frequently consider. Issues, however, will arise in terms of statistical evaluation of efficacy. Obvious ones are (i) how will multiple SNPs be weighted for inclusion in summary statistics and (ii) variable degree of interactions between various SNPs and between a SNP and an external factor.

In order to maximize efficacy, sponsors of NCEs have traditionally frequently promoted higher than optimal doses (Cohen 2001; Cross *et al.* 2002). Not surprisingly, post-approval safety-motivated downward changes in dose schedules are frequent (Cross *et al.* 2002). One major risk is that unless the dose is carefully

selected and matched with the genotype to achieve plasma concentrations within a carefully selected therapeutic window appropriate to each genotype, the advantages from pharmacogenetic targeting may be lost. In principle, recommending higher doses than warranted is equivalent to an otherwise optimal dose in the presence of a metabolic inhibitor or genetic mutation. Therefore, arising from these interindividual differences in pharmacology, areas of regulatory submissions that are likely to attract close regulatory scrutiny are the dose-response studies and the 'standard' dose schedule that is usually recommended. Assessment of the posology of drugs with a continuous response variable depending on the genotype profile will most likely pose a difficult challenge. When the variability is a continuous parameter, specific dose schedules may be required for subsets of individuals. Therefore, when a genotype/phenotype association is shown for a drug metabolized by CYP2D6, as with perhexiline-induced neuropathy for example, potentially four dose schedules may be required for (i) ultrarapid, (ii) homozygous extensive, (iii) intermediate extensive and (iv) poor metabolizer genotypes.

Genotype-related information will need to be communicated to prescribing physicians. This may be relatively easy for phrasing the indication, restricting the use of the drug to those genotype(s) showing the maximum benefit and contraindicating its use in those genotypes excluded from the clinical trials or shown to be susceptible to serious ADRs. It is also probable that special monitoring requirements may have to be recommended for individuals of specific genotype. Drug interaction section would need to emphasize the risks in those with intermediate or poor metabolizer genotype. PMs have no functional enzyme to inhibit or induce but they may require protection from unintended inhibition of alternative pathways of drug elimination. Pharmacogenetics, and its protagonists, promise to revolutionize therapeutics within the next decade. This promise is based on the ability to scan the genome and the presumption of discovering an abbreviated profile of SNPs or haplotype associated with variations in drug responses. Therefore, still looking further in the future, the prescribing information may have to be phrased in terms of not only the drug metabolizing enzymes or pharmacological targets but also in terms of SNPs or haplotypes.

Equally important, however, is the fact that regulatory evaluation of the drug concerned will need to run in parallel with the evaluation of the kit for genotyping. These kits will require approval with their own criteria for approval, especially specificity, sensitivity, positive predictive value and negative predictive value, and specific practical details to be included in their product literature. Physicians will require ready access to rapid genotyping kits or facilities.

In December 2004, the FDA cleared for marketing the first laboratory-based genotyping test system that will allow physicians to consider unique genetic information from patients in selecting medications and doses of medications. The new test is the AmpliChip CYP2D6 genotyping test. Approval for inclusion of CYP2C19 genotype testing followed in January 2005. The new test is the first DNA

microarray test to be cleared by a regulatory authority. It will screen a patient for 31 mutations in the CYP2D6 gene and two mutations in the CYP2C19 gene and is expected initially to cost between US \$300 and 400. It is not intended to be a stand-alone tool to determine optimum drug dosage, but along with clinical evaluation and other tools to determine the best treatment options for patients. The AmpliChip CYP450 Test was launched in Europe in the fall of 2004. However, the physician will need guidance on genotype-related dose adjustments for a variety of drugs metabolized by these isoforms as well as up to date information on potential inhibitors and other substrates of CYP2D6 and CYP2C19. An important issue in the use of this test is who will provide this prescribing guidance and information. For the vast majority of drugs, there are neither the prospective data on the positive and negative predictive values of genotype-phenotype associations nor genotype-related optimal doses.

12. PHARMACOGENETICS AND IMPACT ON FUTURE CLINICAL PRACTICE

Whether the promise of pharmacogenetics is fulfilled remains to be seen (Goldstein 2003; Nebert *et al.* 2003; Tucker 2004). In principle, genotype-based prescribing ought to be more effective at improving response rates and decreasing the socio-economic burdens of ADRs. However, there would have to be a considerable change in drug promotion and prescribing cultures.

An important factor that is likely to limit the potentially beneficial application of pharmacogenetics is the interaction between the genotype and extrinsic factors. Non-compliance by physicians with prescribing information and patients alike is just one of these factors. The over-exalted benefits of pharmacogenetics usually ignore the contribution of these non-genetic factors, which are the more frequent causes of variations in drug responses. A number of drugs have been withdrawn from the market not because of some abnormal pharmacogenetic trait in the patients but because of lack of attention to the prescribing information. For example, nine drugs have been withdrawn from the market over the last decade because of their potential to prolong the QT interval and/or induce TdP. For all these drugs, this unexpected and undesirable response was related almost exclusively to non-genetic factors in the majority of the patients who experienced this ADR. In only 38 of the 341 cases of cisapride-induced ventricular tachyarrhythmias was there an absence of any obvious risk factor (Wysowski *et al.* 2001; Shah 2004). Even physiological states such as menstrual cycle can augment the risk of drug-induced QT interval prolongation.

Drug-drug interactions are another major problem and have frequently resulted in withdrawal of drugs from the market, for example terfenadine, mibefradil, cerivastatin, cisapride and levacetylmethadol. The inhibition of drug metabolizing enzymes by other drugs is an important point of intersection between pharmacogenetics and drug response. An individual of EM genotype can be readily converted into an

individual of PM phenotype by concurrent administration of an inhibitor. For example, quinidine or fluoxetine converts a CYP2D6 EM into a PM and a natural consequence of this iatrogenic 'phenocopying' is that many individuals prescribed 'normal' doses develop high plasma concentrations of the parent drug, exposing them to high dose pharmacology of the drug. To the extent that drugs can inhibit a drug metabolizing enzyme and convert an EM into a PM, the clinical phenotype (drug response) is a moving target and phenocopy is of greater clinical relevance than is the immutable genotype. The presence of co-morbidity often results in aberrant pharmacokinetics and/or altered responsiveness of pharmacological targets. Therefore, much of the benefits of pharmacogenetic targeting of drugs may be lost or more than offset if prescribers do not comply with either the recommendations on pre-treatment genotyping or with safety-related prescribing recommendations (Krasucki & McFarlane 1996; Shah 1999; Smalley *et al.* 2000; Raschetti *et al.* 2001; Curtis *et al.* 2003; Roe *et al.* 2003).

From the sponsors' perspective, the target populations of an NCE whose prescribing is genetically driven would necessarily be smaller than has been the case hitherto. The benefits of pharmacogenetics will also be lost if the doses promoted are inappropriately high, drug interactions are not fully characterized by the sponsors and appreciated by the prescribing physicians or the role of non-genetic intrinsic and extrinsic factors ignored. Unfortunately, evidence available at present suggests that physicians do not take as much notice as they ought to of the prescribing information generally, let alone that related to pharmacogenetics.

In recent times, nowhere are the perils of a high dose, drug interactions, potential role of genetic factors and disregard for prescribing information better illustrated than by cerivastatin, a valuable HMG-CoA reductase inhibitor. Cerivastatin had to be withdrawn from the market within 5 years of its approval because of a large number of reports of fatal and non-fatal rhabdomyolysis. Originally approved at a maximum daily dose of 0.3 mg in June 1997, the recommended dose was progressively increased to a maximum daily dose of 0.8 mg in July 2000. Those individuals who do not show the required efficacy because of the presence of SNPs in the gene coding HMG-CoA reductase (Chasman *et al.* 2004) may have provided the stimulus to this upward pressure on the dose to improve the responder rate. The increment in the benefit was, however, modest. To add to the complexity, cerivastatin is metabolized mainly by polymorphic enzyme CYP2C8 (Backman *et al.* 2002; Wang *et al.* 2002). Two alleles of CYP2C8 (CYP2C8*2 and CYP2C8*3) show diminished drug metabolizing activity. Their frequencies in Caucasians are estimated to be in the range of 0.15. Twenty-three per cent of the 44 patients with rhabdomyolysis in a monotherapy group were taking doses of 0.3 mg or less and a substantial number were associated with the high dose of 0.8 mg daily. It is interesting to speculate whether those patients who developed rhabdomyolysis at lower doses of cerivastatin had impaired CYP2C8 metabolic capacity.

Gemfibrozil markedly inhibits the metabolism of cerivastatin (Backman *et al.* 2002; Wang *et al.* 2002). Of the 31 fatal reports of rhabdomyolysis in association with cerivastatin in the USA, a number were started on high dose at the outset despite an advice to the contrary and 12 were in association with concurrent use of gemfibrozil, despite the combination being contraindicated. Ultimately, on 8 August 2001, the drug was withdrawn from the market worldwide. For very much the same reasons, ADRs and drug interactions have led to the withdrawal of a number of other drugs from the market.

13. CONCLUSIONS

Whether or not the anticipated advances resulting from genome-wide pharmacogenetic studies translate into safe and effective individualized therapy remains to be seen. Discovering highly predictive genotype-phenotype associations during drug development and demonstrating their clinical validity and utility in well-designed prospective clinical trials will no doubt better define the role of pharmacogenetics in future clinical practice. In the meantime, prescribing should comply with the information provided while pharmacogenetic research is deservedly supported by all concerned but without unrealistic expectations.

Although not within the remit of regulatory assessment of drugs in most countries, pharmacoeconomic assessments may well be required to determine the cost-effectiveness of pharmacogenetically driven prescribing of each 'genetically vulnerable' drug on a case-by-case basis (CIOMS 2005). The long-term future of pharmacogenetics in routine clinical practice will no doubt become clearer over the next decade. The important paradigms that will define this future include specificity and sensitivity of the genotyping tests and positive and negative predictive values of the genotype-phenotype associations as well as pharmacoeconomic considerations.

While the role of pharmacogenetics in routine clinical practice awaits better characterization, it seems that education of the prescribing community offers greater and more certain prospects in the immediate future for improving drug therapy and achieving the desired clinical outcomes than may the application of pharmacogenetics.

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