3. Pharmacogenetics and transporters

For the vast majority of drugs, however, the reason for individual susceptibility to ADRs has remained unknown and there are hardly any data on genetic susceptibility. However, recent studies have shown that organ-specific organic anion and cation transporters play an important role in the transport of drugs into the cells. These transporters may account for drug-induced toxicity, hitherto termed "idiosyncratic".

Molecular studies have found evidence of genetic polymorphisms of these transporters in hepatocytes [21, 22]. Mutations in the genes coding for these transporters may lead to dysfunctional polypeptides, which affect not only the pharmacokinetics of the drugs concerned but also the potential hepatotoxic effects of some of these drugs [23, 24]. Furthermore, the variant alleles show inter-ethnic differences [22, 25] that may possibly explain inter-ethnic differences in the hepatotoxic potential of a drug (such as ibufenac). Studies investigating these transporters in patients with hepatotoxicity offer exciting prospects for exploring the potential role of pharmacogenetics in drug-induced hepatotoxicity (see section 5 below).

These transporters and P-glycoproteins co-localise in organs of importance to drug disposition (intestine, liver and kidney). The expression of P-glycoprotein activity is under the control of the MDR1 gene [26] and is an important factor in the disposition of many drugs. In multi-drug resistance (MDR), the processes involved show considerable inter-individual and inter-ethnic variability. For example, a variant allele recently designated as MDR1*2 (resulting from three linked SNPs) occurred in 62% of European Americans and only 13% of African Americans [27].

The MDR1 gene and its variants have significant implications in terms of efficacy or development of resistance to anticonvulsants, antineoplastic therapy and anti-HIV drugs [28, 29].

4. Pharmacogenetics and pharmacological targets

In addition to pharmacogenetic effects on drug metabolism, therapeutically promising examples of genetic variations in pharmacological targets are also beginning to emerge. These targets include receptors, transporters, enzymes, channels and intracellular coupling processes that modulate pharmacodynamic responses. Among the most widely studied are the

pharmacological targets related to cardiac arrhythmias, asthma, depression and the HLA antigen genotype in hypersensitivity reactions.

To date, the focus of pharmacogenetic studies in the context of ADRs has been on drug metabolising enzymes. It is now becoming evident that polymorphisms of pharmacological targets (pharmacodynamic polymorphisms) may in fact be even more important. In one study of 270 cancer patients given antiemetic 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 [30]. In another study by the same group of investigators, patients homozygous for a deletion variant of the promotor region of 5-HTR_{3B} gene were shown to experience vomiting more frequently than did all the other patients [31]. 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 discontinuation. The severity of side effects in paroxetine-treated patients with the C/C genotype was also greater [32]. Thus, although paroxetine is metabolised by CYP2D6, polymorphism of 5-HTR_{2A} is a more important determinant of paroxetine-induced ADRs.

4.1 Polymorphisms of cardiac potassium channels

Drugs prolonging the QT interval of the surface electrocardiogram (ECG) have attracted considerable attention recently. Excessive prolongation of the QT interval, in the right setting, predisposes to torsade de pointes (TdP), a potentially fatal ventricular tachyarrhythmia [33]. The duration of this interval reflects the duration of ventricular action potential. The major determinant of the action potential duration is the potassium current mediated by the rapid component of the delayed rectifier potassium channels (IKr). Many drugs have been withdrawn as a result of their potential to prolong the QT interval and induce TdP.

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. Mutations of the subunits that form these channels, including IKr, are common and give rise to congenital long QT syndromes.

Relatively large numbers of individuals carry variants of long QT syndrome genes that are clinically silent. While these individuals have a normal ECG phenotype, they nevertheless have a diminished repolarisation reserve and are highly susceptible to drug-induced QT interval prolongation and/or TdP following normal therapeutic doses of drugs (such as cisapride, astemizole, terfenadine and halofantrine among others) even in the absence of inhibitors of their metabolism [34]. In an analysis of 341 reports of cisapride-induced ventricular arrhythmias, there were 38 (11%) cases in whom there were no identifiable risk factors or contraindications [35]. These individuals may well represent a population with a concealed genetic defect of their potassium channels.

4.2 Polymorphisms of ß2-adrenoceptors and ALOX-5

Individuals who carry Arg16/Gly16 or Gly16/Gly16 mutations of ß2-adrenoceptors have been shown to respond much less favourably to salbutamol-induced bronchodilatation, in contrast to those with wild type receptor characterised by Arg16/Arg16 genotype – the difference in FEV1 response between Gly16/Gly16 and Arg16/Arg16 genotypes is 6.5-fold [36]. Similarly, asthmatic patients who carry mutations of the core promoter of 5-lipoxygenase (ALOX-5) respond poorly to ALOX-5 inhibitors such as zileuton [37].

Kaye et al [38] have recently shown that in individuals with cardiac failure, patients who were homozygous for the Gln27 allele of the ß2-adrenoceptor displayed a significantly lower proportion of good responders to carvedilol than did patients who were homozygous or heterozygous for the Glu27 polymorphism (26% versus 63%, P=0.003).

4.3 Polymorphisms of the serotonin transporter

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. The insertion variant of this polymorphism (long allele) is associated with higher expression of brain 5-HTT compared to the deletion variant (short allele) [39]. 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 efficacy of fluvoxamine in the treatment of delusional depression has been shown to correlate with 5-HTT genotypes [40].

4.4 Abacavir-induced hypersensitivity reactions and HLA genotype

Hypersensitivity reactions (HSR) to abacavir occur in about 5% of patients who receive the drug for HIV-1 infection. Three independent research groups have identified an association between HLA-B*5701 and hypersensitivity to abacavir in patients of Caucasian ancestry [41-44]; the sensitivity of HLA-B*5701 ranged from 46-94%. While two groups suggest that there may be clinical value in prospectively screening Caucasian patients for HLA-B*5701 prior to the use of abacavir [43, 44], in the largest, and most ethnically diverse study, the association between HLA-B*5701 and hypersensitivity was much weaker in Hispanic patients and was absent in Black patients [45]. While this is an interesting example of the potential of pharmacogenetics, there is legitimate risk that HLA-B*5701 screening could unintentionally compromise the highly successful risk management programme established for abacavir hypersensitivity. Specifically, physician vigilance might be reduced in patients who do not carry markers associated with hypersensitivity and marker-negative patients might be at increased risk for experiencing serious and/or life-threatening hypersensitivity reactions because symptoms associated with abacavir hypersensitivity are not promptly recognised and abacavir discontinued. Efforts to analyse thousands of SNPs across the genome for association to HSR are underway to identify additional genetic markers with sufficient predictive value to be clinically useful [46].

5. Pharmacogenetics and hepatotoxicity

Hepatotoxicity is of serious concern not only because of the morbidity and mortality associated with it but also because it is the leading reason for withdrawal of drugs from the market [47]. This is also evident from inspection of Table 1 in Chapter 2. Apart from the role of transporters at the hepatocytes-biliary canalicular interface, there is conclusive evidence for the role of polymorphic drug metabolism in hepatotoxicity associated with some drugs.

For isoniazid, the genetic basis for this toxicity is well known. Individuals who have a low activity of N-acetyltransferase (NAT2 slow acetylators) are at a much greater risk of developing isoniazid-induced hepatotoxicity. Slow acetylators produce a low level of an intermediate metabolite that is also eliminated by acetylation. Failure to eliminate this effectively results in production of an alternative metabolite that is hepatotoxic [48, 49].

Perhexiline-induced hepatotoxicity, a major factor in the drug's withdrawal from the market, is associated with impaired CYP2D6 status [50]. The involvement of genetic factors in drug-induced hepatotoxicity generally is strongly suggested by the susceptibility of the female gender. In addition, there are reports of familial or ethnic susceptibility to hepatotoxicity associated with some drugs such as phenytoin [51] or ibufenac [52] respectively.

6. Pharmacogenetics and drug interactions

Drug-drug interactions can be dramatically influenced by genotypic differences. A number of studies have shown that 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 to inhibit or induce. Likewise, UMs too may fail to exhibit the expected drug-drug interaction unless the dose of the inhibitor is (toxic) high enough. The individuals most likely to display a drug interaction are those who have an intermediate drug metabolising capacity or those who have inherited CYP2D6 alleles with reduced or altered affinity for CYP2D6 substrates. At the level of CYP2D6, the dependence of drug interactions on the metabolic phenotype has already been shown for a number of its substrates, for example codeine [53], propafenone [54, 55], mexiletine [56], encainide [57], metoprolol [58] and desipramine [59]. The organic ion transporters and P-glycoproteins referred to earlier are additional sites of important drug interactions and pharmacogenetic factors are also likely to be important here.

7. Predictive genotyping: Improving drug response and minimising ADRs

It has been estimated that predictive genotyping (for candidate genes) will lead to benefit in 10-20% of drug treatment by allowing prevention of ADRs [60, 61].

If genetic markers of a greater number of ADRs (candidate genes, SNPs or haplotypes) can be identified and if cheap and rapid genotyping of patients can be done routinely, then the impact of ADRs on morbidity and mortality can be considerably reduced.

Veenstra et al [62] have reviewed cost-effectiveness of genetic tests and have identified five primary characteristics that will enhance the cost-effectiveness of the application of pharmacogenetics. These are:

1. A well-established association between the genotype and drug response
2. The variant gene is relatively common
3. Relatively cheap and rapid genetic test
4. Difficulties in monitoring drug response
5. Severe clinical or economic consequences from not using the pharmacogenetic information

Similar conclusions have been reached by Rioux [63] who has also emphasised the importance of the frequency of the variant allele in determining the cost-effectiveness of the application of pharmacogenetics in therapeutics.

Other workers who have evaluated the potential impact of pharmacogenetics have concluded that its application in therapeutics will be cost-effective "sometimes" and that it would be effective primarily for chronic diseases where unnecessary long-term therapy with an ineffective drug for many years could be avoided in some patients [64].

8. Limitations

It is not intended to suggest that the application of pharmacogenetics will totally eliminate the problems of ADRs. Recently, Kirchheiner et al have provided a preliminary guidance for a number of drugs metabolised by CYP2D6 and CYP2C19 with a view to introducing genotype/phenotype-specific dose schedules [65]. Recommending inappropriately high dose can easily offset the potential benefits of pharmacogenetics. Co-administration of a metabolic inhibitor converts an extensive metaboliser into a poor metaboliser. It is therefore not surprising that drug interactions feature prominently among the causes that lead to withdrawal of drugs from the market.

One unpublished report analysed 17 studies (with a total of about 1,350 patients) published between 1995-2000 on antipsychotic drug therapy, investigating an association between CYP2D6 genotype and both plasma levels of the drug(s) and response to these drugs [66]. There was a relationship between genotype and plasma concentrations of drugs that were predominantly metabolised by CYP2D6 but a large intra-genotypic variability obscured clinical utility of concentration measurements. However, there was no relationship evident between genotype and drug response (i.e. failure to respond beneficially). There was only a modest positive trend between the genotype, especially the presence of

CYP2D6*10 allele in the Japanese, and severity of tardive dyskinesia and extrapyramidal syndrome. This may not altogether be surprising since many neuroleptics have active metabolites. When applying pharmacogenetic testing in routine clinical practice, it is important to take note of the pharmacology of the metabolites relative to that of the parent drug, the fraction of the drug cleared by the polymorphic pathway and the therapeutic index of the drug concerned [67].

In humans, diclofenac is metabolised to 4'-hydroxy (OH), 3'-OH and 5-OH metabolites. The polymorphic CYP2C9 is involved in the metabolism of diclofenac to 4'-OH diclofenac and 3'-OH diclofenac. However, the CYP2C9 genotype does not correlate with diclofenac-induced hepatotoxicity or COX-1 and COX-2 inhibition [68, 69]. Similarly, in asthma, patients who are deficient in 5-lipoxygenase due to a genotypic variant in the ALOX-5 gene are non-responsive to 5-lipoxygenase inhibitors. However, most of the 5-lipoxygenase inhibitor non-responders have normal ALOX-5 genes, and the basis of their non-responsiveness lies in other factors, probably related to the nature of their asthma.

However, if a genotype/phenotype relationship can be shown, pharmacogenetics offers another important strategy by which to reduce ADRs. The dose schedules recommended need to be carefully chosen and the clinical awareness of the consequences of co-administration of interacting drugs need to be heightened. Prior genotyping of patients can be used to improve safe and more effective use of specific and carefully chosen medicines by identifying patients most likely to respond beneficially and those most likely to develop an ADR. This strategy would immediately translate into great reductions in healthcare and economic resources that are currently expended in managing the consequences of ADRs.

Even if a correlation between genotype and phenotype can be established, it is worth remembering that drug-related problem(s) may not be completely eliminated. This is because a number of non-genetic external factors interact with genotype or modulate the response to a drug. In addition, there are a number of other factors that complicate what may appear to be a simple relationship. The reader is referred to Chapter 4 on "Exploring Pharmacogenetics in Drug Discovery and Development" and Chapter 12 on "Unresolved Issues and Barriers to Progress".

9. Conclusions

This chapter highlights the potential contribution of pharmacogenetics in reducing the incidence of dose-related and idiosyncratic ADRs. In relation to ADRs, the research aim of pharmacogenetics is to identify a genetic profile that characterises patients who are more likely to suffer an ADR compared with those in whom the risk is unlikely. Using this knowledge in the clinic, the choice of medicine and dose can be targeted for an individual and the overall result may be an improvement in the safety profile of the drug. Moreover, as a result of improved safety following application of pharmacogenetic principles, improved efficacy may also accrue. Many dosing schedules are limited by appearance of side effects. By eliminating the use of high doses in those genotypes most at risk, it may become possible to evaluate the additional benefits of higher doses in the remaining genotypes.

Advances in biotechnology promise the prospects of characterising genetic variations in individual patients rapidly and cheaply with a view to individualisation of therapy. Exploration of the role of pharmacogenetics should be undertaken during drug development and continued well into the post-marketing period to include the study of rare and delayed adverse reactions. This will make the application of pharmacogenetics in minimising morbidity and mortality from A.DRs a realistic and worthwhile proposition.

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Chapter 4

Exploring Pharmacogenetics in Drug Discovery and Development

1. Introduction

Although defining pharmacokinetic variability has increasingly become a part of phase I drug development, a very limited number of doses are usually taken into phase III development and are based on safety windows for the whole population assuming that all patients are a homogeneous group. The end result is the recommendation of a "standard" dose schedule to be applied to all patients. This practice does not take into account the considerable interindividual variability that exists within the population at large in the dose-concentration-response relationship of a new chemical entity (NCE). Therefore, the consequences of administering a "standard" dose to individuals at either extreme of the variability are all too obvious.

Interindividual variability results directly from interindividual differences in the two key elements of the dose-response relationship of the drug – pharmacokinetics and pharmacodynamics. Interindividual variability in either of these key elements of the dose-response relationship originates from two broad sets of factors – genetic and non-genetic – which need to be placed in perspective in relation to each other. Drug development programmes need to characterise variability generally and the specific contributions of these genetic and non-genetic factors in determining this variability.

1.1 Non-genetic variability

The role of non-genetic factors in pharmacokinetic or pharmacodynamic variability can be significant and arises from the presence of co-morbidity (e.g. hepatic or renal dysfunction), co-administration of drugs that may interact with the index drug or change in internal environment such as endocrine or electrolyte imbalance.

Recognising the pivotal role of pharmacokinetics in determining dose schedules, the effects of co-morbidities and co-medications are almost always explored during drug development. These include the effect of hepatic or renal dysfunction or alterations in the pharmacokinetics of the drug following co-administration of inhibitors or inducers of its metabolism. Other variables that are examined include age, particularly children and the elderly, weight or body mass index and gender. The outcome of

these investigations, when clinically significant, is dose recommendations, contraindications or warnings specific to these variables.

1.2 Genetic variability

Both pharmacokinetics and pharmacodynamics of a drug are strongly influenced by genetic factors as well. The presence of variant alleles often exerts influences that far exceed those due to age, gender or the presence of co-morbidity and co-medications. Genetic polymorphisms of drug metabolising enzymes or pharmacological targets have both been documented to have an impact on variability at a population level and response to drugs resulting in adverse drug reaction (ADR) or failure of response at an individual level.

2. Pharmacogenetic Variability

2.1 Polymorphisms of drug metabolising enzymes

Between 50-60% of drugs undergoing metabolic elimination are metabolised by cytochrome P450 (CYP) drug metabolising enzymes.

It seems probable that all CYP drug metabolising enzymes display polymorphisms, because changes in the DNA sequence are anticipated every 10^2-10^3 bases in all genes. A compilation of human CYP polymorphisms can be found at a website especially dedicated to this at [http://www.imm.ki.se/CYPalleles/]. Although all CYP drug metabolising enzymes have genetic variations, the functional consequences of the majority of these are unknown.

Most of the polymorphisms detected earlier were based on whole or partial gene deletions, mRNA splicing defects or truncation or frameshift mutations. These changes generally lead to non-functioning proteins. This is illustrated by classical polymorphisms such as those of CYP2D6 and CYP2C19. Polymorphisms resulting in amino acid substitutions within protein coding regions of CYP genes can lead to variable functional consequences, ranging from total absence of activity to a protein with altered activity. Again, several examples can be found among variants of CYP2D6 and CYP2C19, but there are an increasing number of functional variants among other CYP enzymes as well, for example CYP2C9.

The presence of a variant in the DNA sequence of an enzyme does not necessarily lead to a functional consequence in the activity of that enzyme. When a change leads to a functional consequence, the outcome depends on the sequence position where the change has occurred. Polymorphisms

in regulatory regions can affect levels of expression of P450s and those in coding sequences may lead to a protein with an altered or absent activity. In principle, the effect is usually expected to be identical in all individuals, but differential interactions with transcription factors and co-activators and repressors may have variable consequences.

Gene amplification of CYP2D6 is also a well-documented phenomenon and frequently results in an "ultrarapid metaboliser" phenotype. A further complication with these polymorphisms is the variable effect on different substrates and reactions. For example, CYP2C9 I359L polymorphism affects warfarin and diclofenac metabolism, but not tolbutamide metabolism, thus highlighting the need to fully evaluate clinical relevance.

A recent trend has been to develop high-throughput methods of scoring single nucleotide polymorphisms (SNPs). However, the problem with this approach is that only a fraction of SNPs have functional consequences. The most direct way to assess the functional significance of a SNP is to express the variant protein in a heterologous system and to study its catalytic properties. Heterologous expression systems also have their limitations as has been shown in the case of thiopurine S-methyltransferase (TPMT) where expression of the mutant variant in a yeast expression system results in normal protein function. In any case the more relevant approach is to determine whether there is an association of the SNP under study and a defined phenotype. Therefore, a more indirect and less certain but complementary approach is to perform large-scale comparisons of SNPs and functions (with probe or other drugs) in clinical trials.

Table 1 provides a broad overview of CYP variant alleles. These polymorphisms can have profound influence on the pharmacokinetics of a drug and the subsequent development programme. The impact of pharmacogenetics in drug discovery, development, regulatory evaluation of an NCE and its post-marketing surveillance is best illustrated by the genetically determined variation in the activity of drug metabolising enzymes such as CYP2D6. Genetic polymorphism in CYP2D6, responsible for oxidation of debrisoquine and a number of cardiovascular and psychoactive drugs, is to date the most widely investigated and best characterised for its clinical implications. Apart from the well-documented studies on perhexiline, anecdotal reports or retrospective candidate gene association studies have shown that individuals with a particular genotype may be at a greater risk of an ADR following administration of some CYP2D6-metabolised drugs (see table 2). This genetically determined probability of an ADR in a small number of indi-

Table 1
Variability in the *in vitro* drug metabolism and number of variant alleles found thus far of CYP enzymes in human liver [See also 1, 2]

Enzyme(s)	"Typical" variation[1] (fold)	Maximal' variation[1] (fold)	Number of variant alleles[2]	Remarks
CYP1A2	8-18	50-100	12 (5 5'-variants)	None well characterised
CYP2A6	23-28	164	15 (3 deletions)	Frequency of deletion variants in Orientals ~15 %
CYP2B6	20	50	6	None well characterised
CYP2C8	>10	large	5 (2 5'-variants)	Changes in paclitaxel metabolism *in vitro*
CYP2C9	5-15	40-100	5	Two exon SNPs (*2,*3): decreased metabolism of some substrates
CYP2C19	7-10	>155	10	Most variants have no enzyme activity. Frequency of PM phenotype in Orientals ~15 %. Ethnic variations
CYP2D6	5-18	>80	about 75	A prototype polymorphism with increased, unchanged, decreased or absent activities. Ethnic variations.
CYP2E1	6-10	20-50	13 (5 5'-variants)	Most not well characterised
CYP3A4	8-15	30-100	24 (6 5'-variants)	Practically none is well characterised
CYP3A5	?	?	At least 11	4 splicing defect variants without any activity *in vivo*

[1] *Fold-variations are approximate only. "Typical" variation refers to values for individuals with no known "extreme" CYP-affecting factors in the history. "Maximal" variation refers to values for individuals with known non-genetic influences (e.g. cigarette smoking, inducers, severe liver disease etc) (See reference 2).*

[2] *According to the CYP allele nomenclature in http://www.imm.ki.se/CYPalleles/Many variants actually contain several nucleotide changes.*

viduals could greatly influence the risk/benefit appraisal of the NCE even at a population level, depending on the clinical consequences of the ADR.

This is hardly surprising given the variability between the genotypes in the pharmacokinetics of a drug that is subject to polymorphic metabolism. Table3 provides a typical estimate of the variability in various pharmaco-

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

Clinical Consequences for the PM	
	Increased risk of toxicity
Debrisoquine	Postural hypotension and physical collapse [3]
Sparteine	Oxytocic effects [4]
Perphenazine	Extrapyramidal symptoms [5]
Flecainide	Possibly ventricular tachyarrhythmias [6]
Perhexiline	Neuropathy and hepatotoxicity [7, 8]
Phenformin	Lactic acidosis [9]
Propafenone	CNS toxicity and bronchoconstriction [0, 11]
Metoprolol	Loss of cardioselectivity [12]
Nortriptyline	Hypotension and confusion [13]
Terikalant	Excessive prolongation in QT interval [14]
Dexfenfluramine	Nausea, vomiting and headache [15]
L-tryptophan	Eosinophilia-myalgia syndrome [16]
Indoramin	Sedation [17]
Thioridazine	Excessive prolongation in QT interval [18]
	Failure to respond
Codeine	Poor analgesic efficacy [19]
Tramadol	Poor analgesic efficacy [20]
Opiates	Protection from oral opiate dependence [21]
Clinical Consequences for the ultrarapid EM	
	Increased risk of toxicity
Encainide	Possibly proarrhythmias [22]
Codeine	Morphine toxicity [23]
	Failure to respond
Nortriptyline	Poor efficacy at normal doses [24, 25]
Propafenone	Poor efficacy at normal doses [26]
Tropisetron	Poor efficacy at normal doses [27]
Ondansetron	Poor efficacy at normal doses [27]

EM = Extensive metaboliser
PM = Poor metaboliser

kinetic parameters due to genetic polymorphism in CYP2D6. Often, the variability is even more dramatic (may be up to 20-fold). It is evident that the exposure to the parent drug is considerably higher in poor metabolisers (PMs) than in extensive metabolisers (EMs).

It is worth noting that even in the absence of CYP2D6 genotyping, when dose is adjusted by measurement of plasma drug concentrations, there have been no clinical problems reported with the use of perhexiline in Australia. This emphasises the critical role of monitoring plasma concentrations of some drugs.

Table 3
Pharmacokinetic consequences of CYP2D6 polymorphism [28]

Pharmacokinetic parameter of parent drug	Consequences for the PM relative to EM *
Bioavailability	2 - 5 fold
Systemic exposure	
Cmax	2 - 6 fold
AUC	2 - 5 fold
Half life	2 - 6 fold
Metabolic clearance	0.1 - 0.5 fold

* EM = Extensive metaboliser

* PM = Poor metaboliser

2.2 Polymorphisms of pharmacological targets

Pharmacogenetic factors also exert clinically significant influences at the pharmacodynamic level; that is at the level of an enzyme, a channel, a receptor, a transporter (of neurotransmitters such as serotonin) or an intracellular coupling process. Among the pharmacological targets that best illustrate the significance of polymorphism are those related to asthma, depression and arrhythmias.

Individuals who carry Arg16/Gly16 or Gly16/Gly16 mutations of the ß2-adrenoreceptors, for example, display a much less favourable immediate bronchodilatory response to salbutamol, in contrast to those with wild type receptor characterised by Arg16/Arg16 genotype [29]. This polymorphism also influences airway responses to regular inhaled ß-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 ± 12.1 L/min lower (p = 0.012) than Arg16/Arg16 patients who had used salbutamol only intermittently as needed [30].

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. The insertion variant of this polymorphism (long allele) is associated with higher expression of brain 5-HTT compared to the deletion variant (short allele). Patients who have one or two copies of the long variant (homozygous l/l or heterozygous l/s) show a better therapeutic response than patients who are homozygous for the short variant (s/s) [31, 32]. The efficacy of fluvoxamine in the treatment of delusional depression has been shown to correlate with the 5-HTT genotypes. Similar data have been reported for other drugs in this class (fluoxetine, sertraline and paroxetine).

Among the arrhythmia-related pharmacological targets studied extensively are the polymorphisms in voltage-gated potassium channels; more specifically those related to congenital long QT syndromes (LQTS). LQTS is a heterogeneous group of disorders, caused by ion channel mutations at 6 different genetic loci at least, resulting in a prolonged cardiac repolarisation, QT interval prolongation on resting electrocardiogram (ECG) and an increased risk of a potentially fatal tachyarrhythmia known as torsade de pointes (TdP). Four of the congenital long QT syndromes, LQT1, LQT2, LQT5 and LQT6, result from mutations of potassium channel subunits, KvLQT1, hERG, minK and miRP1 respectively, while the fourth one, LQT3 results from mutations of the cardiac-specific sodium channel, SCN5A. LQT7 results from mutations of the gene coding for cardiac (and skeletal) inward rectifying potassium channel. LQT4 results from mutation of the gene (ANK2) coding for ankyrin-B, a member of a family of membrane adapters. All these subtypes of LQTS are characterised by diminished repolarisation reserve.

Potassium channels that mediate the outward repolarising current (especially the rapid component of delayed rectifier current) are the targets of class III antiarrhythmic drugs that exert their therapeutic effect by controlled prolongation of the QT interval. Over the last 10 years, many non-antiarrhythmic drugs have attracted considerable clinical and regulatory attention because of their potential to prolong the QT interval. A number of non-antiarrhythmic drugs have been found to have this undesirable activity on cardiac repolarisation and lead to TdP. The primary potassium channel target of a vast majority of these drugs is the hERG subunit. Congenital LQTS is estimated to have a frequency of 1 in 5,000 individuals in the USA (http://www.sads.org/LQTS.html). However, in view of the low penetration of many of the mutant alleles of genes that control the expression of potassium channels, the size of the population with channels that have altered properties or reduced function is substantially larger than that diagnosed by ECG recording alone. While such individuals have a normal ECG phenotype, they have diminished repolarization reserve and are highly susceptible to drug-induced QT interval prolongation and/or TdP, even at the normal recommended doses that are otherwise safe. Studies suggest that up to 15% of cases of drug-induced TdP can be explained by polymorphisms in these genes. The role of genetic factors in drug-induced torsade de pointes is reviewed in detail elsewhere [33]. 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.

Understanding genetic variation in pharmacological targets during drug discovery allows preclinical evaluation of any alterations in the affinity of an NCE for these targets and the clinical response to the NCE that can then be explored and evaluated clinically. This data might be of interest for appropriate patient selection, safety monitoring, or any other factor affecting the future performance of the NCE. For example, NCEs are now routinely evaluated for their affinity to bind to the hERG channel, as this most likely predicts the clinical potential for QT interval prolongation. It is clear, however, that a full functional characterisation of any newly discovered polymorphism in a pharmacological target is required before its full significance for the future development of an NCE can be assessed. Unless the full functional consequences are known, it may be impossible to correlate a genotype with drug response. However, it would be inappropriate to require that full functional significance of a polymorphism be known for a marker to have a utility in guiding drug development and delivery. It is not possible at present to say that we have full knowledge of the drug targets of biomarkers such as lipid levels although their utility in improving healthcare is accepted.

It appears that polymorphisms of pharmacological targets may prove to be more relevant clinically than the polymorphisms of drug metabolising enzymes. 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 discontinuation. The severity of side effects in paroxetine-treated patients with the C/C genotype was also greater [34]. Thus, although paroxetine is metabolised by CYP2D6, polymorphism of 5-HTR_{2A} is a more important determinant of paroxetine-induced ADRs.

3. Pharmacogenetics: Drug development, approval and restrictions

3.1 Termination of drugs from development

Until the completion of the Human Genome Project and the availability of technology to scan the entire genome for SNPs that correlate with

genetically determined drug response, there was (and there still appears to be) a general reluctance within the industry to continue the development of drugs subject to significant genetic variability. For example, predominantly CYP2D6-mediated metabolism of a potential NCE has frequently been seen as a liability ('2D6-liability') and a number of such compounds in 1980s and early 1990s were dropped from further progression. Efforts were instead directed at developing structural analogues of lead compounds that were not eliminated predominantly by CYP2D6-mediated metabolism. A similar situation seems to have now arisen with respect to NCEs that block hERG channels, conferring a 'QTc liability' to the NCE.

Terikalant, a class III antiarrhythmic drug, is metabolised by CYP2D6. It has been shown that the increase in QT interval produced by terikalant correlates well with the degree of impairment in its metabolic elimination by CYP2D6 [14]. The perceived difficulties in managing this genetically driven risk resulted in termination of this compound from further development. There is of course no readily available information on how many other compounds have been dropped during development due to their CYP2D6-mediated metabolism. This approach of discarding polymorphically metabolised candidate drugs has proven resource-intensive and counter-productive, leading to greatly diminished pipeline of innovative NCEs. The application of pharmacogenetics may therefore allow development decisions to be made that will facilitate the progression of NCEs. More recently, metabolism by CYP3A4 is also perceived to be a liability since these drugs (e.g. a number of QT-prolonging drugs or HMG-CoA reductase inhibitors) are highly susceptible to drug interactions.

More recently, the trend seems to be one of developing single isomers of previously marketed racemic drugs in cases where one of the isomers is subject to polymorphic metabolism and introduces wide interindividual variability in AUC at a given dose. This variability can be reduced, with a more predictable efficacy, by eliminating one isomer and administering only the isomer, which is efficacious but less subject to polymorphic metabolism. For example, in case of omeprazole, the ratios of AUC for PMs/EMs of CYP2C19 are 7.5 for (+)-(R)-omeprazole and 3.1 for (-)-(S)-omeprazole [35]. (-)-(S)-omeprazole is now approved and marketed as esomeprazole ('Nexium'). Whether or not this reduction in variability has any clinically relevant practical implications remains to be shown and each drug must be judged on a case-by-case basis. The message here is that drug development programmes should address pharmacogenetics in the context of stereoselectivity in the pharmacology of the NCE when appropriate.

3.2 Pharmacogenetically driven labelling restrictions

In order to comply with various regulatory recommendations, sponsors of NCEs often conduct formal phase I studies in a genotyped panel of healthy volunteers to characterise pharmacogenetic influences on pharmacokinetics. Unfortunately, however, the findings of such studies are rarely carried forward to improving the designs and inclusion criteria of phase II or phase III studies. It is most unusual to see phase II dose-ranging studies that include information on the genotype of the individuals randomised. This omission has serious implications for selecting the most appropriate dose for a pharmacogenetically heterogeneous population in phase III pivotal studies. Ideally, where drugs are metabolised by known polymorphic enzymes, phase II dose-ranging studies should include a wide range of prospectively pre-screened subjects to ensure the inclusion of all the important genotypic subgroups, thus impacting on the efficiency of drug development. It is encouraging to note that there is now a greater trend towards integrating pharmacogenetics in drug development.

In some cases where serious toxicity might have a pharmacogenetic basis, the management of clinical safety of an NCE requires detailed labelling on influences of pharmacogenetic factors. Five drugs best illustrate the current regulatory practice of incorporating candidate gene-based pharmacogenetic information into labels to promote safe and effective prescribing.

Thioridazine is metabolised by CYP2D6 and poor metabolisers of CYP2D6 have high plasma levels of the parent drug. Thioridazine predisposes individuals to excessive QT interval prolongation and torsade de pointes. Therefore, the US Food and Drug Administration (FDA) have now contraindicated the drug in patients known to have reduced levels of cytochrome CYP2D6.

Sertindole, an atypical neuroleptic agent, is primarily cleared by CYP2D6. The PMs utilise an alternative pathway mediated by CYP3A4 for its elimination. Since it is not a routine practice to genotype patients, PMs might be at risk if CYP3A4-mediated pathway was inhibited. Consequently, coadministration of sertindole is contraindicated with ketoconazole and itraconazole, both powerful inhibitors of CYP3A4.

S-citalopram (a potent selective serotonin re-uptake inhibitor) has been approved as 'escitalopram' for depression. It is metabolised predominantly by CYP2C19. The usual dosage is 10 mg once daily, which may be increased to a maximum of 20 mg daily. However, for patients who are known to be poor metabolisers with respect to CYP2C19, the recommendation is to initiate treatment with a dose of 5 mg daily during the first two weeks of treatment. Depending on individual patient response, the dose may be increased to 10 mg daily.

Celecoxib is an orally active, COX-2 selective inhibitor indicated for the symptomatic relief in treatment of osteoarthritis or rheumatoid arthritis. Since celecoxib is predominantly metabolised by CYP2C9, caution is advised when treating patients known to be CYP2C9 poor metabolisers. Fluconazole inhibits CYP2C9 and increases celecoxib mean Cmax by 60% and AUC by 130%. It is therefore recommended that celecoxib be used at half the normal doses in patients receiving fluconazole. Arising from the observed inter-ethnic differences in the pharmacokinetics of the drug, it is also recommended that in black patients, the lower dose (200 mg per day) should be used initially. The dose may, if needed, later be increased to 400 mg per day.

When additional data are or become available, a number of other sections of the prescribing information (e.g. special warnings and precautions for use, drug interactions, ADRs) may need to address the pharmacogenetic profile of potential patients. A recently approved drug that well illustrates this complexity of prescribing information is atomoxetine. This drug, approved by the US FDA in December 2002, is indicated for attention deficit hyperactivity disorder and is metabolised primarily through CYP2D6.

CYP2D6 polymorphism has not only the safety but also efficacy implications. PMs are at risk of a lack of efficacy when the therapeutic effect of a drug is mediated principally by its CYP2D6-generated metabolite. Examples here include codeine and encainide. In particular, PMs exhibit a relative loss of analgesic effects following administration of codeine or tramadol as well as a loss of antiarrhythmic effects following administration of encainide. The therapeutic effects of these drugs are mediated primarily by their metabolites, namely morphine, (+)-M1 metabolite of tramadol and O-desmethyl-encainide (ODE) respectively. In contrast, UMs are at risk from rapidly accumulating metabolites and of poor efficacy when the parent drug mediates the therapeutic effect, for example following administration of normal doses of nortriptyline or perhexiline.

Following results of the Cardiac Arrhythmias Suppression Trial (CAST), the indications for class I antiarrhythmic drugs have been severely restrict-

ed. It is interesting to speculate in retrospect on whether the increased risk of mortality associated with flecainide, encainide or moricizine in CAST may be explained by polymorphic metabolism of these drugs or by mutations of ion channels. Likewise, one may question the role of potassium channel mutations in the observed increase in mortality associated with d-sotalol in the Survival With Oral d-Sotalol (SWORD) study.

If drug response is shown to correlate with a particular SNP(s) or SNPs pattern (haplotype), prescribing information in the future may have to include information on not only in terms of drug metabolising enzymes or pharmacological targets but also in terms of SNP(s) or haplotypes.

3.3 Pharmacogenetics and drug withdrawals

In some cases of serious toxicity, it may not be possible to manage the clinical risk even after careful labelling changes, and a decision has to be made on whether the drug can be allowed to remain on the market. Circumstances leading to the withdrawal of a drug from the market are complex but a conspiracy of genetic factors with other factors (probably the presence of co-morbidity or co-medications) is evident in many drug withdrawals or in termination of clinical development.

The withdrawals of perhexiline and phenformin in late 1980s are almost certainly related to genetically mediated toxicity. Both these drugs are metabolised almost exclusively by CYP2D6 and their clinical uses were associated with serious neuropathy and hepatotoxicity (perhexiline) or lactic acidosis (phenformin). Available evidence strongly incriminates CYP2D6 as a risk factor for both. A number of other older drugs have now been removed from the market. There is a body of evidence which, when viewed collectively, also supports the notion that genetic factors may have contributed substantially to their withdrawal from the market. These drugs include encainide (CYP2D6), terodiline and prenylamine (CYP2D6 and potassium channel mutations) and terfenadine, cisapride and levacetylmethadol (potassium channel mutations).

4. Regulatory framework

It is evident from the foregoing that it is vital to address the influence of pharmacogenetic factors at all stages from research & development (R&D) to post-marketing surveillance of the NCE. Through various guidance notes, regulatory authorities have long articulated their recommendations on the need to address genetic factors during drug development. Not surprisingly, therefore, evaluation of influences of pharmacogenetic factors is also critical during regulatory evaluation and post-marketing clinical use of the drug. The development of an NCE may need to be terminated pre-approval, its labelling highly restricted during approval, or its clinical use suspended post-approval if variability from pharmacogenetics cannot be managed.

Although the requirements to address these genetic factors are stated by different regulatory bodies in different terms, the net effect of the requirements is that new knowledge concerning pharmacogenetic variations in drug response will lead to increased requirements for pharmacogenetic documentation. At present, a number of guidelines from the European Union's Committee for Proprietary Medicinal Products (CPMP), US Food and Drug Administration (FDA) and/or International Conference on Harmonisation (ICH) make direct or indirect references to the need for addressing genetic factors when developing new chemical entities. The guidelines from the CPMP and ICH are shown in Table 4.

Table 4
Pharmacogenetics and CPMP and ICH Guidelines

	Genetic Factors in Pharmacokinetics
1.	Pharmacokinetic Studies in Man [36]
2.	Drug Interactions [37]
3.	ICH - Ethnic factors in the acceptability of foreign clinical data [38]
4.	Bioavailability and Bioequivalence [39]
5.	ICH - Dose-response information [40] *"...metabolic polymorphism..."*
	Genetic Factors in Pharmacodynamics
6.	ICH - Dose-response information [40] *"variability in pharmacodynamic response..."*

The CPMP guideline on "Pharmacokinetic Studies in Man" requires that metabolic studies should indicate whether the metabolism of a drug may be substantially modified in a case of genetic enzyme deficiency and whether, within the dose levels normally used, saturation of metabolism may occur, thereby resulting in non-linear kinetics.

The CPMP guideline on "Drug Interactions" emphasises that subjects participating in metabolic *in vivo* interaction studies should be appropriately

genotyped and/or phenotyped if any of the enzymes mediating the metabolism are polymorphically distributed in the population. In some cases, clinically relevant interactions may only occur in a subset of the total population, for instance, in a PM when an alternative route of metabolism is inhibited or in a heterozygous EM with compromised metabolic capacity.

In April 1997, the US FDA issued a guidance entitled "Drug Metabolism/Drug Interaction Studies in the Drug Development Process: Studies In Vitro" [41]. 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 Koseisho has also issued guidelines in 2001 that recommend genotyping in all drug development programmes for drugs that are metabolised by cytochrome P450s [42, 43].

The ICH guideline on 'Dose-Response Information to Support Drug Registration" recognises that the choice of a starting dose might also be affected by potential interindividual variability in pharmacodynamic response to a given blood concentration level, or by anticipated interindividual pharmacokinetic differences, such as could arise from metabolic polymorphisms or a high potential for pharmacokinetic drug-drug interactions.

It is also recognised by various regulatory guidelines that certain types of ADRs are due to unusual genetically determined pharmacokinetic variations and it is advised that every effort must be made to elucidate the pharmacokinetic mechanisms if there is any reason (e.g. from the knowledge of secondary pharmacology) to suspect that the ADR is caused by the altered pharmacokinetics of the drug.

One important question regarding the demography of a clinical trial population is the extent to which it represents the target population in terms of genetic, pharmacokinetic and pharmacodynamic variability.

Regulatory guidelines acknowledge the importance of inter-ethnic differences in pharmacokinetics and pharmacodynamics of drugs resulting from non-genetic extrinsic factors as well as from global heterogeneity in the frequency of variant alleles of drug metabolising enzymes or pharmacological targets. This global heterogeneity assumes considerable importance now that sponsors often conduct their studies in populations distant from the ultimate target populations. This global development reduces costs, expedites drug development and addresses the issues arising from global prescribing of drugs. The ICH guideline on "Ethnic Factors in the Acceptability of Foreign Clinical Data" recommends that a regulatory submission should include (1) adequate characterisation of pharmacokinetics, pharmacodynamics, dose-response, efficacy and safety in the population of one region and (2) characterisation of pharmacokinetics, pharmacodynamics and dose-response in the new region. The guideline recognises the role of genetic factors and the steepness of the dose-response curve in determining whether a drug is likely to show significant ethnic differences during its clinical use.

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.

The utilisation of genetic information in global development programmes, including bridging studies between populations, will be an area of increasing activity and regulatory interest.

5. Investigating pharmacogenetic influences during drug development

Although the emphasis of the following sections is exploration and characterisation of variability due to genetic factors and genetic influences on drug response, the role of non-genetic factors should not be overlooked during drug development.

5.1 Preclinical studies

During the preclinical phase, a wealth of *in vitro* and *ex vivo* data are generated, which provide direct and indirect indications of possible pharmacogenetic implications for the compound under investigation. The recommendations from the US FDA and the CPMP regarding the design and use of *in vitro* studies for drug-drug interactions make explicit references to pharmacogenetic polymorphisms of drug metabolising enzymes.

With the aid of current *in vitro* approaches, it is possible to tentatively identify very early during the drug discovery and development process the main metabolites and enzymes catalysing the principal metabolic routes of practically any NCE.

In vitro studies provide a direct indication of the participation, or lack thereof, of polymorphic enzymes in the metabolism of an NCE (see table 5), although unqualified extrapolation to clinical setting should not be assumed.

Preclinical data using liver microsomes of course have their own limitations since it is now known that many drug metabolising enzymes are also expressed in other tissues such as the gut wall and these play a substantial role in drug elimination.

The "go/no-go" decision can then be made, based on both qualitative and quantitative information on the role of polymorphic enzymes in the *in vitro* study of an NCE. If it is decided to continue the development of the NCE, these *in vitro* metabolic data provide a rational basis for planning appropriate pharmacological (pharmacogenetic) and clinical studies in a genotyped panel of healthy volunteers and/or patients (for example, with respect to CYP2D6 or CYP2C19) to assess their *in vivo* significance on the kinetics and the dynamics of the compound under study. Observations on the influence of pharmacogenetic factors on the pharmacokinetics of a drug have in the past led to termination of development, restricted labelling or withdrawal of the drug from the market. For these early pharmacogenetic data to be of practical value in terms of labelling and clinical use, it is necessary to show their clinical relevance. Depending on their clinical significance, the labelling can be crafted in terms of indications, dosing regimen, contraindications and precautions or simply providing pharmacological information of interest.

Preclinical studies provide some of the earliest opportunities for investigating the potential of an NCE for drug interactions. For example, in an *in vitro* study of the metabolism of one NCE under development, it was demonstrated that the compound had a high affinity towards CYP2D6 and lesser affinity towards CYP2C19 and CYP3A4 in human liver incubations with CYP-specific probe substrates. On this basis, and correlating with *in vivo* concentrations, it was predicted that the compound might cause *in vivo* interaction with CYP2D6-metabolised drugs, whereas interactions with CYP2C19 or CYP3A4 were less probable. This indeed was later shown to be the case in formal *in vivo* studies. Further, it was demonstrated that the compound was principally metabolised by CYP3A4, with lesser contributions from CYP2C19 andCYP2C9. It was therefore predicted that the compound would show considerable interindividual variability and would be susceptible to CYP3A4 inducers and inhibitors. Indeed, these expectations were also confirmed clinically.

Table 5
In vitro approaches to study metabolism of drugs and new chemical entities for the prediction of participation of polymorphic drug metabolising enzymes

In vitro system	Type of *in vitro* information on an NCE	Usefulness/Problems
Human liver microsomes	* Metabolite pattern and routes * Individual enzyme assignment for each metabolic pathway by selective inhibitors or antibodies * Enzyme kinetics * Interaction studies	* Prediction of variability and interactions at an enzyme level * Prediction of role of polymorphisms * Only phase I and UGT enzymes present
Human hepatocytes Human liver slices	* Metabolite pattern and routes as a function of time * Concentration-dependence of metabolism (kinetics)	* Prediction of role of various pathways to kinetic behaviour, especially those catalysed by polymorphic enzymes * The whole liver enzyme complement expressed in living cells
Recombinant human enzymes Other human organ *in vitro* systems	* Metabolite pattern * Enzyme kinetics * Organ/tissue-specific metabolic and enzyme data	* Assignment of individual enzymes in the metabolism * Prediction of kinetic behaviour in patients with specific organ diseases * Prediction of metabolism in target organs
Humanised transgenic animals (actually *in vivo* system)	* Transgene-specific metabolism (and its consequences)	* If a transgene is polymorphic, prediction to what might happen in humans * A single enzyme in a whole-animal incubation matrix
In silico modelling	* Metabolite patterns * Enzymes participating	* Only as alerts for other studies * Quantitative data still largely not possible
Animal hepatic *In vitro* systems	* Metabolite patterns and activities and participating enzymes	* Comparisons between human and animal data (extrapolation problems).

5.2 Clinical studies

In an attempt to explore the role of pharmacogenetics in determining drug response during drug development, genotyping of all subjects and patients participating in clinical trials is being increasingly considered. The obvious examples are drugs with a very narrow therapeutic index. At present, the development cost of an NCE is estimated to be US$ 802 million. The additional cost of genotyping the entire population in a clinical trials programme would be only a very small fraction of the total cost. This is almost certainly a highly cost-effective investment in terms of the useful information relevant to safety and efficacy of the drug but there may be considerable ethical and practical obstacles.

One alternative strategy worth considering is pre-specified post-hoc genotyping (for relevant drug metabolising enzymes and pharmacological targets) and intensive pharmacologic investigations of individuals of specific regulatory interest. This strategy is illustrated in Figure 1. Arguably, as a proactive measure, the protocol of every clinical trial in man could include a section "Variability in drug pharmacokinetics and pharmacodynamics".

5.2.1 Phase I

Early phase I clinical studies should aim at characterising the effect of genotype on the pharmacokinetics of the drug in healthy volunteers. The role of non-genetic factors such as the influence of co-morbidity (such as liver disease) and co-medications (inhibitors or inducers of drug metabolism) should also be explored and the variability from these non-genetic factors should be compared to that due to genetic factors. In order to characterise the true consequences of genetic variability in pharmacokinetics, it is important to investigate not only the interindividual but also the extent of intraindividual variability. This is best done by studies of replicate design in a panel of genotyped healthy volunteers.

Preclinical and *in vitro* studies should have identified the main drug metabolising enzymes and the potential pharmacological targets (responsible for therapeutic as well as toxic effects) of the parent drug and its main metabolites. If any of these are known to be polymorphic, subjects participating in at least one single dose and one multiple dose studies should be appropriately genotyped and the data analysed for association with any genetic influences in pharmacokinetics or pharmacodynamics. Similarly, subjects in drug interaction studies should be genotyped to ascertain the association of the presence or absence of an interaction with any particular genotype. Genetic influences can be modified or genetic effects reproduced by the presence of co-morbidity. For example, inhibition of a drug metabolising enzyme (e.g. CYP2D6 by fluoxetine) produces a poor metaboliser phenocopy despite an extensive metaboliser genotype.

Intensive pharmacology and pharmacogenetic studies are particularly valuable in those subjects who are pharmacokinetic or pharmacodynamic "outliers" in these phase I studies.

5.2.2 Phase II

Following the above phase I studies in healthy volunteers, it may become necessary to investigate the dose-response relationship in phase II studies in genetically defined subgroups of patients.

These studies should be large enough to include the whole range of variability in drug metabolising capacity. If there is any evidence from preclinical and *in vitro* studies of polymorphic pharmacological targets, consideration should be given to at least one concentration-controlled trial in order to address the issues of polymorphisms in pharmacological targets.

By prospective genotyping, phase II studies should aim at ensuring the inclusion of important phenotype/genotype subgroups so as to allow dosing recommendations appropriate to each genotype, rather than a standard dose schedule to suit all. Pharmacogenetic studies may be particularly valuable in those subjects who are "outliers" in these phase II studies – those who show an exaggerated or much attenuated response to a given dose.

The outcomes of phase I and II studies may influence the prospective design of and dose selection for the pivotal phase III studies.

5.2.3 Phase III

These studies are likely to provide the ultimate evidence on the role of pharmacogenetic factors in determining drug response. Patients with unexpected drug response (in terms of efficacy and safety outliers) should be genotyped appropriately for polymorphic drug metabolising enzymes and pharmacological targets. The responses of interest in this context are failure to achieve any therapeutic benefit or development of concentration-related or other serious ADRs. If an association of either response with a genotype is found, the subjects should be studied intensively for pharma-

cology of the drug in these patients. The increased size of the phase III studies will allow a more definitive understanding of the relationship between genotype and drug response to be established and the benefits of a diagnostic test to be evaluated. Phase III studies also provide further opportunities for investigating the role of non-genetic factors in drug response.

In some instances, however, data generated from phase II studies may suggest inclusion or exclusion of a given subpopulation, for example those with a specific genotype, from the subsequent development programme. However, this enrichment design studies have their own unique problems that must be addressed.

5.2.4 Phase IV

Since not all ADRs are detected during the clinical development of a drug, it is vital that there is effective pharmacovigilance system in place throughout the post-marketing period of the drug. Although logistically complex, it may be valuable to collect blood and/or DNA samples from subjects displaying delayed or rare ADRs in phase IV to allow genotyping and to study any unusual features of the pharmacology of the drug in such individuals.

6. Genotyping versus phenotyping

Although the emphasis in pharmacogenetics is on genotyping of patients, phenotyping is a potentially valuable and at times more effective tool. Patients may be phenotyped for their drug metabolising capacity using appropriate substrate drugs as metabolic probes (e.g. dextromethorphan for CYP2D6). Classification of an individual as either an EM or a PM is based on estimation of drug in the serum at a predetermined time point or of the parent drug and its metabolite in urine sample collected over a defined period. The major advantages of genotyping are that it is unnecessary to have a validated assay for measuring the drug in question, no need to administer a probe drug and the lack of interference from interacting drugs that need not be discontinued. For example, in presence of a metabolic inhibitor of CYP2D6, genotyping a patient will correctly identify an EM whereas phenotyping may result in misclassification of an EM as a (phenocopy) PM. For most pharmacological targets, genotyping is at present the only available option to explore the role of genetic factors. Recently, an epinephrine challenge test has been described as a means of establishing an electrocardiographic diagnosis in silent LQT1 mutation carriers.

7. Maximising the application of pharmacogenetics

The value of applying pharmacogenetics in drug development and routine clinical practice is a complex issue.

The presence of a genetic polymorphism(s) in the path between the administration of a drug and response to the drug does not always adversely affect the risk/benefit ratio even in individuals with genetic mutations. These genetic traits may be of less significance for drugs with wide therapeutic index and/or for drugs with metabolites almost as active as the parent drug.

As for genetic influences on drug response, two models exist - high genetic/low environment versus low genetic/high environment. Genes may be categorised into those that have major, moderate and minor effects. References have already been made above to the confounders arising from drug interactions. Furthermore, application of pharmacogenetics will need to carefully consider the nature of toxicities or the consequences of failure of efficacy. This is in addition to the cost-effectiveness of pharmacogenetic testing. The likelihood of preventing a serious reaction makes pharmacogenetic testing an attractive tool provided the frequency of the variant allele has a critical mass frequency within a population.

Above all, one needs to consider how pharmacogenetics will be applied in routine clinical practice. Availability of reliable and rapid genotyping/phenotyping kits together with physician compliance with prescribing information may prove to be the major determinants of the benefits of pharmacogenetics.

8. Conclusions

It is evident that polymorphisms of drug metabolising enzymes have a profound influence on the pharmacokinetics of the drug of interest. Abnormal pharmacokinetics result in unintentional overdosing of those who cannot metabolise the drug. The converse is true with respect to exposure to metabolites that may be therapeutically active. Polymorphisms of pharmacological targets also result in abnormal or supersensitivity to the pharmacological effects from concentrations that are therapeutic concentrations in the majority of the population.

These polymorphisms may have consequences that adversely alter the risk/benefit ratio of the drug in some individuals, that is those with muta-

tions. It is therefore imperative that the possibility of genetic influences should be considered from the earliest stages of drug development.

If the possibility of a genetic influence arises, its qualitative and quantitative implications should then be explored and characterised at every stage of the drug development. This is especially relevant to phase II dose-finding studies and the selection of dose(s) for pivotal phase III studies.

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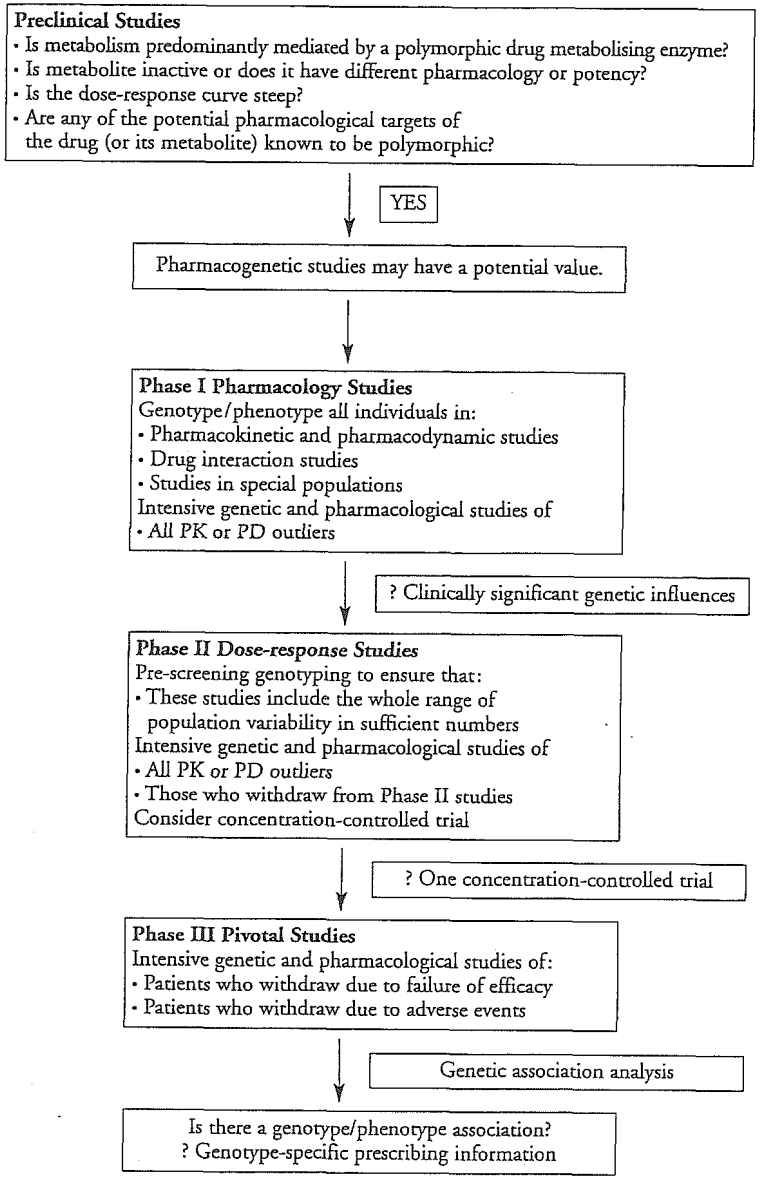
Figure 1
Integrating pharmacogenetics in drug discovery and development



Chapter 5

Impact of Pharmacogenetics on Drug Discovery and Development

1. Introduction

In the last 30 years, the pharmaceutical industry has developed and marketed a large number of medicines that have improved the outcome of many diseases whilst generating significant returns on research and development (R&D) investment for pharmaceutical companies. Over the last 10 years, however, a number of key factors have emerged that impact on the delivery of new medicines to the patient such as:

1. Increase in development costs and time due to greater complexity of clinical development
2. Changing regulatory requirements
3. Increased risk of not getting medicines to market as attrition rates in development are increasing
4. Increased risk of medicines not remaining on the market as safety concerns have caused the withdrawal of a number of medicines in recent years
5. The need to model potential impact on clinical, societal and economic aspects of the treatment to the satisfaction of healthcare providers

The progress and the refinement of research tools, combined with the ever increasing societal demand for safer and more effective medicines, continue to fuel the high cost of development of each new medicine.

Consequently the return-on-investment per drug is decreasing, and perhaps more importantly, the flow of new medicines to patients has gradually diminished. Thus pharmaceutical companies need to use all available tools in order to overcome this situation, with pharmacogenetics currently offering a significant potential. Although still at a basic and experimental stage, pharmacogenetic data are already being submitted to regulatory authorities. A recent CMR survey [1] reported nine companies having experience of submitting applications to the authorities that included pharmacogenetic and pharmacogenomic data, with pharmacogenetic data being included in 4 investigational new drug (IND) applications, 4 clinical trial (CT) applications and 1 new drug application (NDA).

In this chapter, we focus on the contribution of genetic variations to understanding variability in drug response. It is recognised that some of