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Chapter 2

Abnormal Drug Response (I): Clinical, Social and Economic Burden

1. Introduction

From the very beginning of monitoring drugs for their safety, attention has been paid to the economic consequences of adverse drug reactions (ADRs) [1-3]. ADRs have long been recognised as a significant cause of morbidity and mortality but the true extent of the problem has remained a matter of discussion and informed speculation. Almost a quarter of a century ago, Mach and Venulet [3] considered methodological issues for estimating the economic aspects of ADRs, and calculated direct and indirect costs using several case scenarios.

Prescribing the most effective drug in individual patients is more often than not a process of trial and error. Therefore, in addition to ADRs, failure of efficacy of a drug also imposes significant burdens. However, data quantifying the healthcare and economic impacts of patients failing to respond to the medicines prescribed first time are sparse.

The most common ADRs are dose- or concentration-related (type A) pharmacological reactions that account for about 75-80% of all ADRs. These include reactions related to prescription of inappropriate drug or inappropriate doses of a drug as well as drug interactions. Usually, clinically relevant drug interactions result in an increase in plasma concentration of one of the interacting drugs to toxic levels. Other common types of ADRs are immunologically-mediated (type B for bizarre, idiosyncratic or hypersensitivity reactions). Classification of ADRs has also included those termed type C (following continuous or chronic use), type D (that are delayed such as carcinogenic or teratogenic effects) and type E (end-of-use ADRs that result from withdrawal of a drug; "rebound phenomenon"). Recently, ADRs of type F have been added to this increasingly complex classification and these result from unexpected failure of therapy.

As early as 1972, it was estimated that 6.9 to 22% of all ADRs are in fact due to drug interactions [4]. Although the majority of drug interactions result in pharmacokinetic changes with no clinical consequences, about 1 in 7 drug interaction studies, submitted to the US Food and Drug Administration (FDA) during the period 1992-1997, led to changes in

labelling, the majority of which involved dose adjustments [5]. One review of the available studies suggests that up to 30% of hospital patients and 70% of ambulatory patients could be receiving potentially interacting drugs [6]. Drug interactions are increasing and are now recognised as a frequent cause of hospital admissions [7-10]. The number of drugs withdrawn recently because of their interaction potential and clinical consequences testifies to this increasing problem, resulting from (generally unintentional) polypharmacy.

There are a large number of international studies that estimate the scale of the clinical burden due to ADRs. Others have attempted to quantify the social and economic consequences of ADRs in terms of healthcare availability, resource implications and gross national productivity. All those involved in the development and use of medicines, whether they be payers, pharmaceutical companies, patients, physicians, or regulators agree that ADRs are associated with suffering and costs.

This chapter reviews a sample of representative studies on the overall impact of ADRs and failure of efficacy.

2. ADR-related morbidity and mortality

2.1 ADRs in community medicine

In one of the earliest studies assessing the impact of ADRs, Mulroy reported that 1 in 48 consultations in general practice in the UK was due to an ADR [11]. A study by Lumley et al estimated that 0.8% of all general practitioner consultations are directly due to ADRs [12]. Following a survey of 817 patients and using a much broader definition of ADR, Martys reported that 41% of the patients in general practice have had a reaction to the drug prescribed [13].

More recent studies from France have estimated an incidence of about 2 adverse effects per general practitioner per day [14] and 2.6 cases of serious ADRs per general practitioner per year [15]. Despite the enormous progress in therapeutics since the late 1970s, the incidence of ADRs has not changed [16-20].

2.2 Drug-related hospital admissions

Using data compiled prior to 1977, Venulet reported the incidence of ADRs in already hospitalised patients as ranging from 2 to 18% [21]. A review in 1993, based on 36 English-language studies of ADRs leading to hospital admissions, reported that on average, 5.5% of all hospital admissions are due to ADRs [22]. The incidence varied from 0.2% to 21.7% depending on the population under investigation.

In an Italian hospital, 235 of 5,497 patients who visited the emergency department over a 1-year period (October 1994 to September 1995) did so because of an ADR. Of these, 45 were hospitalised. Dose-related therapeutic failures (55.6%) were the main cause of drug-related admissions whereas ADRs (63.8%) caused the most frequent drug-related visits. Although drug interactions accounted for only 3.8% of the visits, their consequences were more severe, and most of these patients had to be hospitalised [23].

The percentage of hospital admissions due to drug-related causes, including ADRs and therapeutic failures, has been variously estimated to be 11.4% in Denmark (study period was March 1988 to May 1989) [24], 13.8% in Sweden (September 1997–October 1998) [25] and 5.7% in Australia (November 1994–December 1994) [26]. In one study of 452 admissions to a university hospital in the USA, 16.2% of the admissions were considered drug-related which included 8.8% due to drug therapy failure (July 1993–August 1993) [27].

The percentage of hospital admissions specifically due to ADRs has been estimated at 8.4% in Denmark [24], 7.5% in the UK [28], 3.3-7% in Switzerland (from 1996 onwards) [29, 30], 3.2-7.2% in France (March–April 1998 in one of the studies) [31, 32], 2.7% in Australia [26] and 2.4% in Germany (October 1997–March 2000) [33]. The most recent study (November 2001 to April 2002) reported a 6.5% prevalence of ADR-related hospital admissions in two major hospitals in the UK [34].

In the US, the overall incidence of serious ADRs was computed to be 6.7% on the basis of a meta-analysis of 39 prospective studies from hospitals [19]. Of these, 2.1% had occurred in patients while in hospital and 4.7% were present in patients requiring admission as a result of ADRs. Seventy-six per cent of ADRs were Type A dose-dependent. Another meta-analysis of studies confirmed the heterogeneity of the published data. However, these studies do consistently emphasise the considerable proportions of all hospital admissions that are related to ADRs. Larger studies have shown lower percentages although the elderly were reported to be at a 4-fold greater risk. Beijer and de Blaey reported

that 88% of the ADR-related admissions in the elderly and 24% in the non-elderly were preventable [35].

2.3 Drug-related mortality

The data on drug-related or ADR-related mortality are complicated by the heterogeneous nature of the studies but they do provide an estimate of the problem.

Shapiro et al reported as long ago as 1971 that as many as 160,000 deaths resulted from ADRs each year in US hospitals [36]. The overall evidence from a number of recent studies suggests that 0.3-0.5% of deaths are related to ADRs.

In England and Wales, the number of deaths related to ADRs has risen steadily over the last 10 years. One UK study of 3,277 Coroner's Inquests during 1986 to 1991 identified 10 deaths due to prescribing errors and another 36 deaths caused by ADRs [37]. These 46 deaths accounted for approximately 1 in 2,000 of all the deaths during the study period. A prospective 6-month study from Norway reported 1% drug-related mortality among 3,082 hospitalised patients [38]. Only 2 of these were recognised as drug-related by the attending clinicians. This gross under-recognition of ADR-related mortality is supported by another study from US that compared the number of deaths attributed to ADRs on death certificates with data in the spontaneous post-marketing surveillance system of the FDA (MedWatch) during 1995. During this period, 206 deaths were certified as being due to ADRs, whereas the MedWatch tabulated 6,894 fatalities [39]. It is recognised that the fatal outcomes recorded in MedWatch are not necessarily causally drug-related. However, this 34-fold variation must be a matter of concern.

ADR-related mortality was reported to be 1% in the UK [28] and among 4,331 hospital admissions, 0.18% in Switzerland [29]. ADRs were estimated to be between the fourth and sixth leading cause of death in the USA; the fatality rate as a result of ADRs amongst the hospitalised patients was 0.32 % [19]. Pirmohamed et al reported an overall mortality rate of 0.15% due to ADRs [34].

3. Healthcare burden

In terms of time spent in the hospital, it is not surprising that a patient with an ADR spends longer time in a hospital and consequently, imposes greater economic burdens on the healthcare systems.

3.1 Duration of hospitalisation

Mean duration of hospital stay was 15.1 days for each of the 10 patients with an ADR and 10.7 days for those without an ADR in one study from France (conducted during May 1993–October 1993). In the same study, the mean stay was 19.2 days for the 21 patients in whom the ADR occurred in the hospital [40]. Other studies have estimated the duration of hospital stay at 13 ± 10.6 days in Germany (October 1997–March 2000) [33] and 10.6 days for patients with ADRs and 6.8 days for matched controls in the USA (August 1998-December 1998) [41]. ADR-related excess stay in hospital was computed at 7.6% of all hospital days in France [40] and 5.9% of all emergency beds in Australia [26]. For the 1,225 ADR-related hospital admissions in the UK, the median duration of hospital stay was 8 days [34].

3.2 Drug-related hospitalisation costs

Estimates on costs of ADRs leading to hospitalisation are complicated by geographical differences in healthcare costs and a lack of common units of measurement and methodologies.

The cost of ADRs leading to hospitalisation was estimated at Euro 11,357 per hospital bed per year in France [32] while a study from Switzerland estimated a mean cost per case at Swiss Francs 3,586 or a total of Swiss Francs 821,204 over the 6-months study period [30]. In the US, the cost of hospitalisation was US$ 22,775 per case for patients with an ADR and US$ 17,292 per case without an ADR [41]. In Australia, the annual cost for all drug-related admissions was estimated at just under A$ 3.5 million (comprised of A$ 1.63 million for unavoidable, A$ 1.67 million for avoidable and A$ 0.2 million for definitely avoidable admissions) [26]. The cumulative direct costs for hospitalisation over the 30-month study period in Germany were estimated to be Euro 4 million in the two urban study areas and the annual direct cost for the whole country was estimated to be Euro 400 million [33]. In the French study above, about 5-9% of hospital costs were related to ADRs [40]. When Pirmohamed et al extrapolated their findings to the entire National Health Service in the UK, the projected annual cost of ADR-related admissions was estimated to be £466 million [34]. Others had previously estimated these costs in the UK to be in the range of £1.5-2.6 billion [42].

Lazarou et al [19] estimated the direct hospital costs due to ADRs in the US to be US$ 1.6-4 billion. Ernst and Grizzle [43] updated their previous 1995

estimate of US$ 76.6 billion for the annual cost of drug-related morbidity and mortality resulting from drug-related problems in the ambulatory setting in the United States to reflect treatment patterns and costs in 2000. They estimated that in 2000, the mean cost for a treatment failure was US$ 977 per patient. For a new medical problem, the mean cost was US$ 1,105, and the cost of a combined treatment failure and resulting new medical problem was US$ 1,488. Overall, the cost of drug-related morbidity and mortality in the US exceeded US$ 177.4 billion in 2000. Hospital admissions accounted for nearly 70% (US$ 121.5 billion) of total costs, followed by long-term care admissions, which accounted for 18% (US$ 32.8 billion).

4. ADRs and pharmacovigilance

4.1 Costs of pharmacovigilance

Pharmacovigilance, or activity and programmes to detect and monitor ADRs, and efforts to reduce and prevent ADRs each incurs significant costs. These costs include administration of national and global monitoring systems (e.g. the Yellow Card Scheme in the UK or the MedWatch Scheme in the US), changes in prescribing information, dissemination of this information and in extreme cases, withdrawal of drugs.

An indirect estimate of costs of ADRs may be obtained by examination of the benefits of Bar Code Regulations issued by the FDA in February 2004 [44]. The preliminary estimate of the cost for implementing this bar coding is thought to be between US$ 0.5 billion and 1.4 billion over a 10-year period. The purpose of bar coding is to ensure accurate identification of medications, and thereby reduce medication prescribing errors, and ultimately, mortality and morbidity. As stated above, one study estimated these at more than US$ 177 billion including US$ 121.5 billion in hospital costs and US$ 32.8 billion in long-term care expenses [43].

4.2 ADRs and drug withdrawals

Drug withdrawals are costly for the companies. Worldwide there were 121 safety-related drug withdrawals between 1960 and 1999. Market life was known for 87 of these. About 31% of these products were withdrawn within the first two years and up to approximately 50% were withdrawn within the first five years [45].

In the UK, a total of 583 new active substances (NAS) were approved between the years 1972 and 1994 and of these, 59 were later withdrawn.

This represents a withdrawal rate of 2.57 NAS per year over this period [46]. Thirty-four drugs have been withdrawn from various markets for safety reasons over the 15-year period from 1990 to 2004 and have included a number of high profile drugs as shown in Table 1.

Table 1
Drugs withdrawn from various markets (1990 to 2004) for safety reason

Drug	Year of withdrawal	Reason(s) for withdrawal from market
Dilevalol	1990	Hepatotoxicity
Triazolam	1991	Neuropsychiatric reactions
Terodiline	1991	QT interval prolongation and TdP (TdP = torsade de pointes)
Encainide	1991	Proarrhythmias
Fipexide	1991	Hepatotoxicity
Temafloxacin	1992	Hypoglycaemia, haemolytic anaemia and renal failure
Benzarone	1992	Hepatotoxicity
Remoxipride	1993	Aplastic anaemia
Alpidem	1993	Hepatotoxicity
Flosequinan	1993	Excess mortality possibly due to proarrhythmias
Bendazac	1993	Hepatotoxicity
Soruvidine	1993	Myelotoxicity following drug interaction
Chlormezanone	1996	Hepatotoxicity and severe skin reactions
Tolrestat	1996	Hepatotoxicity
Minaprine	1996	Convulsions
Pemoline	1997	Hepatotoxicity
Dexfenfluramine	1998	Cardiac valvulopathy and pulmonary hypertension
Fenfluramine	1998	Cardiac valvulopathy and pulmonary hypertension
Terfenadine	1998	Drug interactions, QT interval prolongation and TdP
Bromfenac	1998	Hepatotoxicity following prolonged administration
Ebrotidine	1998	Hepatotoxicity
Sertindole	1998	QT interval prolongation and potential for TdP
Mibefradil	1998	Statin-induced rhabdomyolysis following drug interaction and concerns on other potential drug interactions, including the risk of TdP
Tolcapone	1998	Hepatotoxicity
Astemizole	1999	Drug interactions, QT interval prolongation and TdP
Trovafloxacin	1999	Hepatotoxicity
Grepafloxacin	1999	QT interval prolongation and TdP
Troglitazone	2000	Hepatotoxicity
Alosetron	2000	Ischaemic colitis
Cisapride	2000	Drug interactions, QT interval prolongation and TdP
Droperidol	2001	QT interval prolongation and TdP
Levacetylmethadol	2001	Drug interactions, QT interval prolongation and TdP
Cerivastatin	2001	Rhabdomyolysis following drug interactions
Rofecoxib	2004	Myocardial infarction and strokes

(Tdp = torsade de pointes)

The withdrawals of perhexiline (an antianginal drug) and phenformin (an oral hypoglycaemic agent) 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) and lactic acidosis (phenformin). Available evidence strongly incriminates CYP2D6 as a risk factor for both. For a number of other older drugs now 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). Although the costs of developing new drugs are difficult to estimate precisely, overall costs have been estimated at approximately US$ 400 million in 1998 and US$ 800 million in 2001 [47, 48]. Although these are overall costs and include the costs of failures during early development, they do indicate the substantial loss of investment due to ADRs.

Drug withdrawals deprive patients who did not suffer from ADRs of the benefits of the medicine. For example, following the withdrawal of terodiline in the UK (one of the three major markets of this drug), the regulatory authority in the UK received representations from a number of patients and physicians to make this drug available, albeit on a named patient basis. Similar demand had followed the withdrawal of perhexiline, an antianginal drug that was highly effective in patients who did not respond to other drugs and were not suitable for coronary artery bypass surgery.

5. ADRs and litigation

ADRs inflict additional burdens on healthcare resources through litigations. One study by Kelly [49] identified 1,520 significant adverse drug events published in ClinAlert during the period 1976 to 1997. Of these, 56% (n = 846) were life-threatening, 29% (n = 447) resulted in death and 15% (n = 227) resulted in permanent disability. Litigation was reported in 14% of fatal cases of ADRs and the settlement averaged US$ 1.1 million. Other data from this study [50-52] relevant to this report are summarised in the Table 2.

Table 2
Analysis of adverse drug events published in ClinAlert during the period 1976 to 1997 - adapted from Kelly WN [49]

	All serious	Life-threatening	Fatal	Permanent disability
Adverse drug **events** cases identified	1,520 (100%)	846 (55.7%)	447 (29.4%)	227 (14.9%)
Adverse drug **reactions**	52%	50%	58%	43%
Type A reactions	19%	7%	34%	9%
Type B reactions	61%	93%	66%	91%
Setting where drug started:				
- Hospital	67%	89%	56%	57%
- Out-patient	29%	5%	41%	38%
Usual recommended dose in	73%	82%	64%	43%
Common drug classes				
- CNS	24%	26%	24%	16%
- CVS	10%	11%	12%	5%
- Oncology	11%	7%	17%	15%
Litigation				
- Reported in	13%	1%	14%	56%
- Mean settlement	US$ 3.1 m	US$ 1.1 m	US$ 1.1 m	US$ 4.3 m

Claims and litigation are an additional burden on healthcare. In the UK National Health Service, these amounted to £400 million in paid litigation in 1998/99 with an expected potential liability of £2.4 billion. In countries such as India, the inclusion of the medical profession under Consumer Protection Act has resulted in ever increasing litigation and malpractice suits [53].

Private litigations against pharmaceutical companies have also increased, as seen in class actions related to dexfenfluramine ("fen-phen" leading to primary pulmonary hypertension and cardiac valvulopathy) and cerivastatin (leading to rhabdomyolysis). The potential liability from such class actions usually runs into billions of dollars. In the US, the sponsor of dexfenfluramine had taken charges related to "fen-phen" related litigation of US$ 13.2 billion, an amount estimated to be sufficient to cover the overall funding requirements [54]. With regard to cerivastatin, the lawyers had stated that the compensation could total around US$ 800 million, related to just the fatal cases alone. Given that the total number of all potential claimants is thought to be more than 4,000, it has been estimated that settlement could reach US$ 5 billion [55].

6. ADRs and indirect costs

Indirect costs are those sustained by the community as a result of ADRs. They arise from the loss of individual contribution to the gross national product (GNP). This loss in GNP is related to (a) the excess time spent in the hospital, (b) the time taken by the individual to fully recover from an ADR (usually a serious one) to the point when (s)he can return to previous work, (c) the time taken by the individual's family member(s) to care for him or her and (d) social benefits paid to the individual while off work.

These indirect costs may vary enormously and can amount to hundreds of thousands of dollars, particularly in cases in which the ADR results in permanent disability [3]. There is a great need to review and further develop methodology for assessing these indirect costs.

7. Conclusions

ADRs and other drug-related problems result in considerable clinical morbidity and mortality. They account for a significant proportion of hospital admissions and such patients generally spend longer time in the hospital. Consequently, the direct implications for healthcare and economic resources are considerable. Indirect economic costs and social burdens are difficult to compute but estimates suggest that these may be comparable if not even greater.

A number of valuable drugs have had to be withdrawn from the market as a result of the clinical risks they posed. Withdrawal of these drugs also has consequences for those patients in whom they are effective.

Since the majority of ADRs are dose- or concentration-related, they may be preventable, or at least reduced, by paying careful attention to factors that may increase plasma concentrations. Non-genetic factors such as inappropriate doses or drug interactions may possibly be controlled in the majority of cases. Increasingly, however, many ADRs appear to have a genetic substrate. It seems likely that genetic influences resulting in pharmacodynamic variability may be even more important than those resulting in pharmacokinetic variability [56].

The present agenda for "value for money" in healthcare provides the impetus to better quantify the problem and develop measures that minimise human and healthcare costs. These measures include reduction in the frequency or prevention of ADRs.

Given the advances in pharmacogenetic technology, there is a pressing need to study systematically whether pharmacogenetics can help minimise further the burdens of drug-related problems. This requires at least 'preliminary' evidence indicating that many drug-related problems may in fact have a pharmacogenetic basis.

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Chapter 3

Abnormal Drug Response (II): Opportunities for Risk Reduction Through Pharmacogenetics

1. Introduction

An adverse drug reaction (ADR) can result from a variety of risk factors including variability in pharmacokinetics and pharmacodynamics of a drug due to the genetic make-up of an individual. Other important influences are external factors such as co-medications and co-morbidities, which give rise to drug-drug or drug-disease interactions. The net effect of these interactions is that the prescribed dose of a drug is an inappropriate one. Usually, clinically relevant drug interactions result when the plasma concentration of one of the interacting drugs increases to toxic levels.

With careful attention to prescribing information regarding dose, age-related adjustments and populations at risk for drug-drug and drug-disease interactions, the impact of ADRs can be greatly minimised. However, it is unlikely that any single approach will completely eliminate all ADRs. With available data suggesting that some ADRs might have a monogeneic or polygeneic basis, the application of pharmacogenetics provides an opportunity for further reductions in both the incidence and severity of ADRs.

This chapter reviews some of the data on abnormal drug response related to polymorphisms in drug metabolising enzymes, pharmacological targets and drug transporters. It illustrates how, at least in some areas, pharmacogenetics may offer the prospects of minimising the risks of drug toxicity and therapeutic failures.

2. Pharmacogenetics and drug metabolising enzymes

A number of drug metabolising enzymes displays genetic polymorphisms. Candidate gene association studies, investigating the role of these polymorphic drug metabolising enzymes such as CYP2D6, CYP2C9, CYP2C19, N-acetyltransferase (NAT2), thiopurine S-methyltransferase (TPMT), UDP-glucuronosyltransferases (UGTs) and dihydropyrimidine dehydrogenase (DPD), have already shown that there is a genetic predisposition to a number of ADRs.

It is now generally assumed that because of this genetic predisposition, there may be a great potential for preventing ADRs and improving the

safe and effective use of medicines through the increasing knowledge of genetic factors that determine drug response. Polymorphic genes and products of gene expression have been considered as markers for optimisation of drug therapy, most especially in the field of oncology.

2.1 Polymorphic variation in CYP2D6

Studies over the last two decades have shown that any given population may be divided into two phenotypes – extensive metabolisers (EMs) or poor metabolisers (PMs) – depending on their ability to mediate CYP2D6-dependent hydroxylation of the antihypertensive drug debrisoquine. Among the EM phenotype, there are two subgroups of particular interest at either extreme of the EM population distribution. One subgroup, termed the ultrarapid metabolisers (UMs), is comprised of individuals possessing multiple copies of the gene for normal metabolic capacity and the other group, termed the intermediate metabolisers (IMs), is comprised of a heterozygous genotype ("gene-dose effect"). UMs metabolise drugs so avidly that they attain very low concentrations of the parent drug and high concentrations of rapidly accumulating metabolites while IMs display a modest impairment in drug metabolising capacity.

CYP2D6 is responsible for the metabolism of well over 60 drugs that include antiarrhythmics, ß-adrenoreceptor antagonists, antihypertensives, antianginals, neuroleptics, antidepressants, analgesics as well as a number of other miscellaneous drugs. Candidate gene association studies have shown that a number of ADRs to CYP2D6 substrates are related to CYP2D6 genotype (Table 1).

One of the first reports on the clinical significance of CYP2D6 polymorphism and its association with serious toxicity was perhexiline-induced neuropathy in patients with impaired CYP2D6 metabolism. Although the recommended dose of perhexiline was 100mg three times daily, a recent study of 23 patients has shown that to maintain the plasma concentrations of perhexiline within the therapeutic and non-toxic range, PMs required a dose of 10-25 mg/day while EM and ultrarapid EM required 100-250 and 300-500 mg/day respectively [1]. Other clinical consequences for individuals with the PM or ultrarapid phenotypes of CYP2D6 are also shown in Table 1.

Application of pharmacogenetic principles may also improve efficacy. There are several examples where subjects carrying certain alleles suffer from a lack of drug efficacy because of ultrarapid metabolism caused by multiple genes or by induction of gene expression. As with perhexiline,

some patients who are ultrarapid metabolisers fail to respond to conventional doses of nortriptyline and require 'megadoses' of this antidepressant. Similarly, poor metabolisers fail to respond to therapeutic effects mediated by metabolites. This is illustrated by the relative loss in PMs of analgesic effects following administration of codeine or tramadol or the loss of antiarrhythmic effects of encainide.

2.2 Polymorphic variation in CYP2C9

Retrospective case studies have shown that the presence of mutant CYP2C9 allele (especially CYP2C9*3 allele) confers a significantly increased risk of bleeding following treatment with warfarin. Available

Table 1
Clinical consequences for PM and ultrarapid EM phenotypes of CYP2D6

Clinical Consequences for the Poor Metaboliser	
	Increased risk of toxicity
Debrisoquine	Postural hypotension and physical collapse
Sparteine	Oxytocic effects
Perphenazine	Extrapyramidal symptoms
Flecainide	? 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
	Failure to respond
Codeine	Poor analgesic efficacy
Tramadol	Poor analgesic efficacy
Opiates	Protection from oral opiate dependence
Clinical Consequences for the Ultrarapid Metaboliser	
	Increased risk of toxicity
Encainide	? Proarrhythmias
Codeine	Morphine toxicity
	Failure to respond
Nortriptyline	Poor efficacy at normal doses
Propafenone	Poor efficacy at normal doses
Tropisetron	Poor efficacy at normal doses
Ondansetron	Poor efficacy at normal doses

data, however, indicate that although the CYP2C9*3/CYP2C9*3 genotype is associated with dramatic over anticoagulation soon after the introduction of oral anticoagulants, overdose during the maintenance period is mostly related to environmental factors [2, 3]. It is also recognised that interindividual variability in warfarin sensitivity also originates from environmental factors. In one study, age and CYP2C9 genotype accounted for 12% and 10% of the variation in warfarin dose requirements, respectively [4]. Clearly, other pharmacodynamic (such as to an abnormality in the target enzyme vitamin K epoxide reductase) and dietary factors also play an important role. In a retrospective cohort study of patients on long-term warfarin, it was found that the mean maintenance dose varied significantly among the six genotypes of CYP2C9. Compared to patients with the wild type genotype, patients with at least one variant allele required longer time to achieve stable dosing and had a significantly increased risk of a serious or life-threatening bleeding event, although patient numbers were small for some genotypes in this study [5].

Similarly, to achieve a therapeutic serum concentration of phenytoin, patients carrying at least one mutant CYP2C9 allele required a mean phenytoin dose that was about 37% lower than that in patients with wild type genotype (199 mg/day versus 314 mg/day) [6]. Since phenytoin has a narrow therapeutic index and genotyping may be carried out rapidly and at a relatively low cost, dosage adjustment based on CYP2C9 genotype, especially at the induction of therapy, would be of value in order to lower the risk of concentration-dependent phenytoin toxicity in the carriers of mutant alleles.

2.3 Polymorphic variation in CYP2C19

CYP2C19 mediates the major pathway responsible for metabolic elimination of proton pump inhibitors. Since therapeutic activity correlates with exposure to the parent compound, it is not surprising that a number of studies have shown that PMs of CYP2C19 respond better to *H. pylori* eradication therapy. These preliminary findings need to be confirmed in large prospective studies [7]. EMs of CYP2C19 require higher doses of these drugs.

2.4 Polymorphic variation in thiopurine S-methyltransferase

Azathioprine and 6-mercaptopurine are metabolised by thiopurine S-methyltransferase (TPMT). The activity of TPMT is inversely related to the risk of developing acute leucopenia associated with the use of these drugs. A number of studies have shown that the risk of azathioprine-induced acute leucopenia can be greatly reduced by basing the initial azathioprine dose on TPMT genotype or phenotype [8, 9]. Of course, not all azathioprine-induced toxicities have a genetic basis. In one study of 93 patients, it was noted that azathioprine-related gastrointestinal side effects are independent of TPMT polymorphism [10]. The value of genotyping for TPMT is illustrated by a report from Murphy and Atherton [11] that by initiating therapy at dose levels of 2.5-3.5 mg/kg in atopic eczema patients with a normal TPMT level, they felt confident in reducing the frequency with which tests of bone marrow and liver function had to be undertaken.

2.5 Polymorphic variation in UDP-glucuronosyltransferases

Conjugation reactions such as glucuronidation mediated by UDP-glucuronosyltransferases (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, and lead to genetic polymorphisms [12, 13]. Some polymorphic UGTs have demonstrated a significant pharmacological impact in addition to being relevant to drug-induced ADRs. Two major isoforms of UDP-glucuronosyltransferase, UGT1A1 and UGT1A9, have been shown to display genetically determined wide interindividual variability in their activities. Studies investigating the role of UGT1A isoforms in the metabolism of drugs such as irinotecan [14, 15], flavopiridol [16, 17], tranilast [18] and atazanavir [19] have been most valuable in explaining the safety issues (myelosuppression, diarrhoea or hyperbilirubinaemia) associated with the use of these drugs.

A meta-analysis by Phillips et al [20] identified 131 specific drugs, 55 drug classes, and 19 therapeutic drug categories as being associated with ADRs. All except three of these drugs were included among the top 200 selling drugs in the United States. The therapeutic categories associated with the most common ADRs were cardiovascular, analgesics, psychoactive drugs and antibiotics. This meta-analysis included 18 of 333 ADR studies and 22 of 61 variant allele review articles. It identified 27 drugs frequently cited in ADR studies. Among these drugs, 59% were metabolised by at least one enzyme with a variant allele known to cause poor metabolism. In contrast, only 7% to 22% of randomly selected drugs were metabolised by enzymes displaying genetic polymorphism ($p = 0.006 - < 0.001$). These data suggest that drug therapy based on the genotype of individual patients may result in a clinically important reduction in adverse outcomes.

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