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# Genetic determinants of dose optimisation: molecular biology in the prevention of drug toxicity

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## Abstract

Adverse reactions to drugs (ADRs) are a major clinical problem, and can be divided into two types (A and B). Genetically determined inter-individual variability in drug metabolism is an important determinant of type A ADRs, particularly for drugs with a narrow therapeutic index. Examples include 6-mercaptopurine and warfarin, which are metabolised by thiopurine methyltransferase and *CYP2C9*, respectively. Whether such findings can be incorporated into clinical practice in a cost-effective manner requires investigation in prospective randomised clinical trials. Predisposition to type B ADRs is dependent on multiple genes interacting with environmental factors. If the genetic predisposition to a type B ADR is known, then dose optimisation may simply entail complete avoidance of the drug. Pharmacogenomic approaches using SNP mapping may provide the opportunity to prevent ADRs in the future; a major limiting factor, however, will be the need for adequate numbers of well-characterised patients in order to have studies with the required statistical power. In summary, molecular biological approaches have, to date, been of limited influence in preventing ADRs. However, with our increasing knowledge of the human genome, coupled with the development of high-throughput genotyping methods, there is a potential to prevent ADRs through individualisation of drug choice and dosage. © 2001 Elsevier Science B.V. All rights reserved.

*Keywords:* Adverse drug reactions; Pharmacogenetics; Polymorphisms

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## 1. Introduction

Adverse drug reactions (ADRs) continue to be a major clinical problem, accounting for many deaths, as well as being a drain on resources. For example, at least 5% of all hospital

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admissions are due to ADRs [1]. A meta-analysis has suggested that in the USA in 1994, ADRs were responsible for over 100,000 deaths making them between the fourth and sixth commonest cause of death [2]. Furthermore, adverse drug events are associated with an increased length of stay in hospital of 2 days, and an increased cost of approximately US\$2500 per patient [3,4].

Current clinical practice involves the administration of the same drug to different patients with the same disease, often at the same dosage, i.e. one drug fits all. The clinical response in this situation can be one of three types:

1. The patient responds with either cure or control of the disease process, i.e. the drug shows the intended therapeutic effect.
2. The patient does not respond either in terms of efficacy or toxicity, necessitating a change in therapy, or alteration in dose (usually an increase in dose). The subsequent response again may be unpredictable.
3. The patient develops an adverse reaction to the drug, which may be fatal, or require specific and symptomatic treatment, or at the very least, require discontinuation of the drug and change in therapy.

Clearly, this is not an ideal way of managing patients, and has led to the concept of personalised medicines, and the birth of the term pharmacogenomics [5,6]. This can be defined as the study of the genetic basis for the differences between individuals in responses to drugs in order to tailor drug prescriptions to individual genotypes. The purpose of this review is to evaluate the role of molecular biology, and in particular, our increasing knowledge of the genetic basis of the variation in drug responsiveness, may play in preventing drug toxicity, and thus improving the benefit-risk ratio of currently available and newly developed drugs.

## **2. Classification of adverse drug reactions**

From a clinical perspective, ADRs can be divided into two broad types, type A and type B [7]. Their characteristics are shown in Table 1. Type A reactions are predictable from the known pharmacology of the drug and often represent an exaggeration of the known primary and/or secondary pharmacology of the drug. Given their dose dependency, they may be particularly amenable to dose titration. In contrast, type B ADRs are bizarre reactions that are unpredictable from the known pharmacology of the drug, and show no apparent dose–response relationship. Therefore, dose titration may be difficult or impossible, and the only alternative available may be avoidance of the drug. Crucial to this argument is the lack of a dose–response relationship; this is discussed in greater detail below.

Typically, type A ADRs have been labeled as host-independent, i.e. not dependent on genetic factors. This is clearly an over-simplification, since there is now increasing evidence of the role of genetics in determining drug disposition and drug response, and with it, susceptibility to ADRs. In general, predisposition to a type A ADR will be dependent on one gene, or one gene may be the predominant factor with a number of other

Table 1  
 Characteristics of type A and type B adverse drug reactions

Characteristic	Type A	Type B
Dose-dependency	Usually shows a good relationship	No simple relationship
Predictable from known pharmacology	Yes	Not usually
Host factors	Genetic factors may be important	Dependent on (usually uncharacterised) host factors
Frequency	Common	Uncommon
Severity	Variable, but usually mild	Variable, proportionately more severe
Clinical burden	High morbidity and low mortality	High morbidity and mortality
Animal models	Usually reproducible in animals	No known animal models

genes contributing to a smaller extent. In contrast, type B ADRs have always been labeled as being host-dependent, although there has been little evidence as to what this host dependency entails. However, recent research suggests that type B ADRs may be similar to complex human diseases in having a polygenic predisposition. This may make detection and prevention more difficult than for type A ADRs.

Type A ADRs account for 80% of all adverse reactions [8]. Because they are common, type A ADRs are usually detected during the pre-clinical phases of drug development. In contrast, type B ADRs are usually only detected during post-marketing surveillance (phase IV), since during pre-marketing, only 1500–2000 patients will have been exposed to the drug, and thus the studies will not have had adequate power to detect the rare forms of drug toxicity.

### 3. Sources of variation in drug response

There are many sources of variation in drug response in the human population. In keeping with the principles of pharmacology, they can be divided into pharmacokinetic and pharmacodynamic factors (Table 2). To date, most of the interest has centred around pharmacokinetic factors, and in particular, on the role of drug metabolism. However, with the increasing realisation of the role of transporters in drug disposition, and of receptor variation in determining drug response, there has also been increasing interest in identifying the genetic determinants of the variation in these areas.

For type A reaction, only one of these sources of variation may be responsible for predisposing to the ADR. In such cases, identification of the source of variation, and subsequent delineation of the dose–response relationship may allow dose titration and thus, prevention of the ADR. With type B ADRs, there may be multiple sources of variation, and each of these may have a different dose–response relationship, which may render dose titration very difficult, if not impossible. However, identification of

Table 2

Sources of genetic variation in the human body that may act as predisposing factors for adverse reactions

Source of variation	Example of protein implicated	Example of drug affected
<i>Pharmacokinetic</i>		
Absorption	<i>P</i> -glycoprotein	Digoxin
Distribution	Alpha <sub>1</sub> -acid glycoprotein	Indinavir
Metabolism	Cytochrome <i>P</i> 450 2D6	Perhexilene
Excretion	<i>P</i> -glycoprotein	Digoxin
<i>Pharmacodynamic</i>		
Receptor	Ryanodine receptor	Halothane
Ion channel	Potassium channel	Clarithromycin
Enzyme	Butyrylcholinesterase	Suxamethonium
HLA	HLA DR4	Hydralazine
Cytokine	Tumour necrosis factor-alpha	Carbamazepine

the sources of variation for a particular drug may be important in itself since it may lead to prospective identification of at-risk individuals and thus avoidance of the drug in those individuals.

It is also important to note that although Table 2 refers to genetic sources of inter-individual variability, environmental factors such as disease, alcohol, smoking and diet may also be significant sources of variability, and may indeed be predominant. These also need to be taken into account when determining optimum doses for prevention of drug toxicity.

#### 4. Type A adverse drug reactions

To date, the focus in the prevention of type A ADRs has been on the role of drug metabolism. However, more recently, the importance of drug transporters in overall drug disposition, has become increasingly appreciated, and it is likely that there will be significant findings in this area over the next few years, which will undoubtedly have an impact on dose optimisation in the prevention of ADRs. Indeed, it is now becoming routine practice during new drug development to screen drugs for their potential to act as substrates for transporters such as *P*-glycoprotein.

Undoubtedly, one of the major successes of molecular biology in the field of drug metabolism has been the elucidation of the genetic basis of variability in drug metabolising enzymes. This has also led to the development of cell lines expressing specific *P*450 isoforms together with their allelic variants that can be used as screening tools during the early phases of drug development. Such in vitro screens for polymorphisms in drug metabolism provide an early stage decision-making tool for the medicinal chemist. Information obtained from such studies will determine whether it would be profitable to genotype individuals in phase I, phase II and even phase III studies. The impact of a particular polymorphism in drug metabolism on drug response, and type A adverse drug reactions, is a function of fractional clearance by the polymorphic enzyme, pharmacological activity of the metabolites and the therapeutic index of the drug [9].

The identification that a particular drug is subject to polymorphic metabolism in *in vitro* screens may lead to subsequent studies in genotyped panels of volunteers and patients. The feasibility of such an approach will depend on the gene frequency, genetic penetrance and the magnitude of the gene–drug interaction. Thus, the impact of variants of *CYP2D6* can be studied prospectively in panels of genotyped individuals. However, when the frequency of the variant allele is lower, for example for *CYP2C9*, it may be not possible to recruit adequate numbers of individuals homozygous for the variant allele. Such *in vivo* studies, however, may be crucial to define the role of the polymorphic enzyme in the metabolism of the drug, and to predict the magnitude of the change in *in vivo* clearance of the drug (determine fractional clearance).

Where the type A ADR is largely dependent upon polymorphisms in genes coding for a particular enzyme that is a rate-limiting determinant for the clearance of the drug, there is a potential for such genetically determined ADRs to be detected early in the drug development process by the use of simple *in vitro* screens. This now represents a critical decision in the early design and development of the drug, and is now seen as part of drug discovery. It may be possible to redesign the drug in order to eliminate the effect of the polymorphism (with the caveat that certain advantages may be lost and other problems may occur). Alternatively, the decision can be to go forward and place some restrictions in the SPC on the use of the drug, or advice on dosing, particularly when the drug is to be used for a disease where there are few or no other available therapies.

In such a circumstance, at least in theory, molecular biology could have a major impact on dose optimisation. However, this has not always been borne out in clinical practice, where polymorphic metabolism is largely ignored. Perhaps the two most important examples where clinical practice largely ignores polymorphic metabolism are drugs metabolised by *CYP2D6* and *N*-acetyl transferase-2 (NAT-2).

The *CYP2D6* polymorphism is perhaps the most extensively studied polymorphism in the field of drug metabolism. *CYP2D6* plays a role in the metabolism of 25% of all prescribed drugs [10]. The rate of drug metabolism can be 100-fold greater in “extensive metabolisers” than in “poor metabolisers”. Approximately, 6% of the Caucasian population carry two null alleles at the *CYP2D6* gene locus [11]; such individuals will have complete loss of enzyme activity and can be easily identified by the use of simple DNA-based tests. However, there is no provision for genotyping patients for *CYP2D6* polymorphisms in clinical practice in the great majority of hospitals, and thus no dose optimisation. Indeed, if the current and historical substrates are analysed, only rarely has polymorphic metabolism by *CYP2D6* been implicated as a major factor in determining the survival of the drug on the market [12]. Examples include phenformin (lactic acidosis), perhexilene (hepatotoxicity and peripheral neuropathy) and terodiline (ventricular arrhythmias). For most other substrates, such as haloperidol, their use is not contingent on prospective typing for *CYP2D6*. A similar story exists for NAT-2, which is absent in 50% of Caucasians [13]. There are number of drugs where the rate of acetylation is a determinant of the occurrence of toxicity. For example, isoniazid is well known to predispose to peripheral neuropathy and possibly SLE in slow acetylators [14]. However, despite its widespread use as first-line therapy for TB, there is no provision for either phenotyping or genotyping prior to starting drug therapy. It is possible that many patients who are deficient in these drug-metabolising enzymes may be suffering unnecessarily

from being given these polymorphically metabolised drugs. Why is it that we do not phenotype or genotype patients before the use of such drugs? Possible reasons include the fact that (a) procedures to type patients are not routinely available, (b) typing procedures are expensive, and (c) typing of patients has not yet been shown to be cost-effective.

Perhaps another determinant of whether patients are genotyped prospectively may be the fact that most of the drugs metabolised by *CYP2D6* and NAT-2 have a relatively wide therapeutic index, and thus most of the reactions are not serious. In these circumstances, therefore, cost effectiveness will be the major determinant of whether a test is done before drug therapy. In contrast, where the toxicity is more severe, for example where the drug has a narrow therapeutic index, prospective genotyping may be more acceptable to clinicians, particularly if it is evidence-based. Two drugs, 6-mercaptopurine and warfarin, merit further discussion in this context.

#### 4.1. 6-Mercaptopurine and thiopurine methyl transferase

Thiopurine methyl transferase (TPMT) catalyses the conjugation of the methyl group from *S*-adenosylmethionine to aromatic and heterocyclic thiol groups. TPMT is involved in the metabolism of 6-mercaptopurine (6-MP), and its pro-drug azathioprine [15]. TPMT exhibits a trimodal distribution of phenotypes: at least eight allelic variants associated with low enzyme activity have been identified at the TPMT gene locus [16], in addition to the presence of an inactive pseudogene [17]. At least 10% of Caucasians have intermediate activity (i.e., are heterozygotes), while 1 in 300 inherit TPMT deficiency [16]. Patients with TPMT deficiency can develop fatal haemopoietic toxicity with full doses, while a reduction in dosage by 90–94% can lead to successful treatment without such toxicity [18,19]. In contrast, patients with wild-type alleles may require higher dosages to ensure efficacy in the treatment of acute lymphoblastic leukaemia [20]. A biochemical assay of erythrocyte lysates is currently used to assess TPMT activity [16,21]; however, spurious results can be obtained when patients have been given blood transfusions, a frequent occurrence in this group of patients. There is, therefore, a need to develop robust genotyping methods, for example with DNA chip technology, which are able to detect not only those patients with the common *TPMT*\*2 and *TPMT*\*3 alleles, but also patients with rare mutant alleles [16]. TPMT is thus a clear example of a clinically significant genetic polymorphism where prospective genotyping may allow individualisation of drug therapy and thereby maximise efficacy and minimise toxicity.

#### 4.2. Warfarin and *CYP2C9* allelic variants

The role of genetic variation in the metabolism of warfarin by *CYP2C9* has attracted a great deal of attention recently. Polymorphisms in the *CYP2C9* gene result in at least two allelic variants. The two most widely studied include *CYP2C9*\*2, where cysteine substitutes for arginine at position 144 and affects binding of *P450* reductase, and *CYP2C9*\*3, where leucine substitutes for isoleucine at residue 359 in the substrate binding site [22]. Both allelic variants result in enzymes that have decreased catalytic activity towards a number of substrates, including *S*-warfarin, the more potent enantiomer of the most widely used oral anticoagulant in clinical practice.

Decreased clearance of warfarin by both allelic variants has been shown in vitro [23,24]. Recently, it was shown in a Japanese population that clearance of *S*-warfarin is reduced in vivo in heterozygotes and in homozygotes (although only one homozygote was studied) [24]. Clinically, these variants have been shown to be associated with a reduced warfarin dose requirement, greater difficulty in initiating warfarin treatment, and an increased risk of bleeding [25]. In a subsequent study [26], using a larger cohort of patients ( $n=561$ ), the relationship between *CYP2C9* genotype and warfarin sensitivity has been confirmed by another group; however, in contrast to the study by Aithal et al. [25], it was found that possession of an allelic variant did not increase susceptibility to severe over-anticoagulation. On the basis of a small pilot study ( $n=38$ ) [27], it has more recently been suggested that the relationship between genotype and clinical phenotype might be further refined by typing for the *CYP2A6*\*3 allele.

It must be stressed that a number of other factors may confound this relationship. First, the anticoagulant response is partly dependent on *R*-warfarin, which is metabolised by *CYP1A2* and *CYP3A4* [28]. Second, there are a number of pharmacodynamic factors, such as vitamin K status and thyroid disease, which alter sensitivity to anticoagulants. Third, there are rarer mutations in the clotting factors such as prothrombin that may alter sensitivity to warfarin [26]. Fourth, there are other methods of dose titration and dose maintenance with warfarin, for example prescribing by computer program [29] or home monitoring [30], which have been shown to be more effective than conventional prescribing. Finally, the clinical use of warfarin dictates that the genotype of the patient would be required within 24 h of admission. Therefore, it may be premature to recommend that all patients should be genotyped prior to taking warfarin. Before this can become a routine part of clinical practice, there is a need for a prospective randomised clinical trial, which not only incorporates into its trial design the different methods for monitoring and altering warfarin dosage, but also the confounding factors mentioned above.

## 5. Type B adverse drug reactions

Predisposition to type B adverse drug reactions is thought to be multi-factorial involving many genes interacting with environmental factors [8]. In a similar fashion to complex diseases [31], it is likely that there is going to be heterogeneity and that different combinations of gene variants give rise to a similar phenotype. Furthermore, preliminary evidence indicates that the frequency of any polymorphism contributing to the phenotype will be only slightly elevated in the disease group when compared with unaffected controls. Thus, prevention of these ADRs is going to be difficult, and will require collection of DNA samples from well-characterised cohorts of patients with defined toxicities. Given the rarity of some of these reactions, it is essential that multi-centre international collaborations are set up to ensure that any studies have adequate statistical power (the problem of samples size and statistical is addressed below).

Type B ADRs have been characterised as being dose-independent (Table 1), or rather there is no simple relationship between dose and the occurrence of toxicity [32]. Certainly, evaluation of patients with and without hypersensitivity to a particular compound shows very little difference in doses received, and indeed in the patients with hypersensitivity,

the doses may have been lower since the drug had to be withdrawn. Furthermore, even within the hypersensitive group, there is little relationship to the occurrence and severity of toxicity and the dose administered. However, intuitively, there must be some kind of dose–response relationship since if the patient had not received the drug they would not have developed the hypersensitivity reaction. Since many type B ADRs are thought to be mediated by the formation of chemically reactive metabolites through metabolism by P450 enzymes (a process termed bioactivation) [32], perhaps a relationship exists with the “internal dose”, i.e., the concentration of the toxic metabolite formed in the body (Fig. 1). However, since these metabolites by definition are unstable, it has not been possible with the currently available technologies to evaluate the dose–response relationship. The situation is further compounded by the fact that the different sources of variation in the human body (Table 2) may all have a different dose–response relationship. Nevertheless, evidence for the existence of such a dose–response relationship can be gleaned from clinical situations where different doses have to be given to the same group of patient in different circumstances. For example, in HIV-positive patients, the anti-infective agent co-trimoxazole has to be given at low doses for prophylaxis against *Pneumocystis carinii* pneumonia (PCP) (960 mg once daily), while for acute treatment of PCP, much higher doses (up to 8 g/day) may be administered. The frequency of hypersensitivity reactions is lower with the prophylactic dose (30%) than with the acute dose, where rates as high as 80% have been reported [33,34]. Given that co-trimoxazole hypersensitivity is thought to be mediated by the toxic nitroso metabolite of sulphamethoxazole [35], one of the

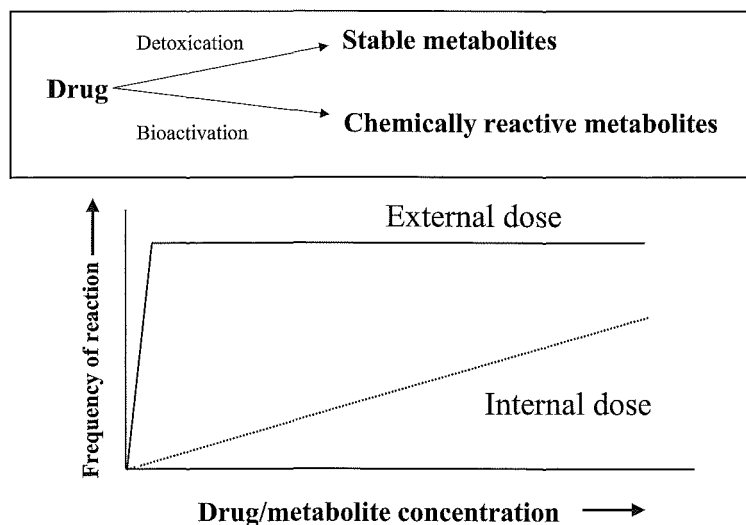


Fig. 1. Hypothetical diagram illustrating the relationship between dose (external and internal) and the occurrence of type B adverse drug reactions. The box shows that drugs can be converted to both stable metabolites (detoxication) and chemically reactive metabolites (bioactivation). The latter have been implicated in the pathogenesis of type B adverse drug reactions. Thus, although type B reactions do not show any relationship with the external dose, i.e. the dose of the drug administered, there may be a relationship with the concentration of the chemically reactive metabolites (referred to as internal dose).



components of co-trimoxazole, this may reflect different concentrations of nitroso metabolite being formed *in vivo*. This is a plausible hypothesis, but one that needs further investigation.

In the absence of definitive evidence for a dose–response relationship with type B ADRs, dose optimisation may simply entail complete avoidance of the drug. The problem of the multi-factorial nature of type B ADRs can be illustrated with respect to work carried out in our laboratory on carbamazepine hypersensitivity.

### *5.1. Carbamazepine hypersensitivity*

Anti-convulsants produce a hypersensitivity syndrome in which the skin is again the major target organ [36]. Skin biopsy data have shown the involvement of cytotoxic T-cells and pro-inflammatory cytokines such as TNF- $\alpha$  [37]. There is both clinical and biochemical data which suggests that this form of idiosyncratic toxicity has a genetic basis [38,39]. *Ex vivo* studies have shown that cells from hypersensitive patients are more susceptible to the toxic effects of drug metabolite(s) generated *in situ* [36,40]. However, genetic analysis failed to reveal an association with known polymorphisms in the enzymes for drug bio-inactivation in man [41,42].

We are therefore exploring polymorphisms in genes associated with events downstream from drug metabolism in the pathogenesis of the skin reactions, in particular the TNF- $\alpha$  gene. This cytokine was so-named because of its ability to shrink tumours [43]. It is a transmembrane (26 kDa) protein cleaved by a specific metalloproteinase to a mature 17 kDa protein that circulates as a homotrimer, and binds to its receptors (p55 and p75). It has a vast range of physiological and pathophysiological effects. A number of polymorphisms have been detected in the promoter region of the TNF- $\alpha$  gene including –238 (G  $\rightarrow$  A) and –308 (G  $\rightarrow$  A) polymorphisms. These have been shown to act as predisposing factors for a number of infectious and inflammatory disorders [44,45]. In our patient group, we found an association between the –308 polymorphism and serious, but interestingly not non-serious, hypersensitivity reactions to carbamazepine (Pirmohamed *et al.*, unpublished data). No association was demonstrated with the –238 polymorphism. Demonstration in an independent sample population is required to confirm this association. However, we have shown a biochemical rationale for TNF- $\alpha$  in the pathogenesis of the hypersensitivity reactions, and have thus satisfied two out of the three criteria laid down by Todd [46] to define a relationship between a clinical phenotype and a SNP. However, such studies take many years, because of the difficulty in obtaining sufficient numbers of clinically homogeneous samples. Such an endeavour can only be undertaken once the drug is being used by tens of thousands of patients, *i.e.* at the postmarketing stage, and is therefore out with the drug development programme.

## **6. Future perspectives**

The current approach to identifying genetic predisposition to ADRs is limited by our knowledge of the mechanisms of the ADR, and thus our restricted choice of candidate genes. An alternative strategy is that based on on-going efforts to develop a comprehen-

sive, densely spaced, genome-wide single nucleotide polymorphism (SNP) map which may allow us in the future to conduct screens for pharmacogenetically active genes as whole-genome, unbiased searches [47]. SNPs are single-base differences in the DNA sequence, observed between individuals, which occur throughout the human genome at a frequency of about 1 per 1000 DNA base pairs. The vast majority of SNPs are biologically silent, but some do have functional consequences. Although SNP-based association studies can be performed by testing of SNPs with functional consequence, this will be limited by our knowledge of their function. An alternative and more powerful strategy is to use SNPs as markers of linkage disequilibrium; it has been estimated that a whole genome scan may require testing of 30,000 SNPs [31]. It is important to note that most SNPs arise on the haplotypic background of other variant alleles, and indeed it has been shown that the use of one or two SNPs is insufficient to detect most associations, even if the SNPs themselves fall on associated haplotypes [48]. This has been re-iterated by a recent study of SNPs of the human beta<sub>2</sub>-adrenoceptor gene which showed that complex interactions of multiple SNPs within a haplotype affected the biologic and therapeutic phenotype, and that individual SNPs may have poor predictive power as pharmacogenetic loci [49].

A high-density SNP map can be used to correlate clinical information from patients with, and without, ADRs. This can be used to identify the responsible alleles that lie in close physical proximity to the SNP by linkage disequilibrium. In theory, the SNP information alone could be used to predict individual patients at risk of ADRs by creating individual SNP profiles [47]. Taken together with knowledge of the underlying mechanisms of the ADR, it may then be possible to define doses that may be associated with efficacy but not toxicity.

Given the need to test for multiple markers simultaneously, an issue that needs to be considered is the sample size and the level of statistical significance required to prevent detection of false-positive associations. A recent study has suggested that for testing 100,000 loci in a genome-wide screen will require a threefold greater sample size at a significance level of  $2.5 \times 10^{-7}$  [50]. This does suggest that for pharmacogenomic detection of rare adverse events, testing in phases I–III is not likely to be practical, and will require prospective storage of samples and evaluation in phase IV when a problem has been identified.

## **7. Conclusions**

It is fair to say that molecular biology has had little impact on the prevention of ADRs to date. Perhaps the most important contribution has been the delineation of the molecular basis of deficiency or reduced activity of the drug metabolising enzymes, which has led to the development of expression systems and their use as screening tools during the pre-marketing phases of drug development. The knowledge gained from such studies, which is often combined with data from *in vivo* studies in genotyped panels of volunteers, is of practical use since it can be used to inform clinicians on prescribing in the summary of product characteristics.

Despite well-known polymorphisms in many drug metabolising enzymes that have been implicated in the pathogenesis of ADRs, it is not routine clinical practice to genotype

patients prior to drug prescription. This may be a reflection of the lack of clinical studies that have shown the clinical utility in adopting such an approach. The need for prescribing by genotype may be greatest when the drug has a narrow therapeutic index, for example, warfarin or drugs used in cancer chemotherapy. However, there is an urgent need to perform prospective randomised clinical trials that are designed to determine whether prescribing by genotype is both clinically and cost-effective. Ultimately, with the advent of SNP profiling, molecular biology could have a large impact on the prevention of type A adverse drug reactions through dose optimisation.

In contrast to type A ADRs, type B ADRs do not have a simple dose–response relationship, and thus, dose optimisation may simply entail avoidance of the drug. However, before we reach this stage, research is required to (a) determine the relationship between the concentration of any toxic metabolites and the occurrence of toxicity, and (b) delineate the multi-factorial and heterogeneous nature of type B ADRs. They are therefore likely to pose an even greater challenge than the type A ADRs. Many serious adverse reactions are uncommon and currently only identified in the post-marketing phase. A major limiting factor in pharmacogenetic prediction of type B reactions is going to be the limited numbers of patients, and therefore, the statistical power of the studies. Thus, despite the obvious advances in genetics, it is likely that type B reactions will not be prevented in the near future, and any investigation of their genetic predisposition will be limited to the phase IV stages of drug development, as is the current situation.

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### **Appendix A. Discussion 16**

**A. Bye:** From the drug industry, we cannot benefit from these things until phase IV. The question is: how do we see the surrogate development for these effects? On one of your slides, it was the classic pathology-type approach where you produce sick patients or reproduce this in animals and then examine post mortem tissues etc. Why don't we start looking in living systems for these changes, which are probably most critical to see round about the 10th to the 14th day? I just wondered if you had any insights into the surrogates that we might be chasing.

**M. Pirmohamed:** At the moment, we have very limited data on surrogates that we need to chase, but this is where molecular biology does come in, and we can use molecular biology to be able to define those surrogates, or bio-markers. We need to be able to first of all look at the molecule; Kevin Park showed a nice slide with particular areas of a molecule that may be important in producing chemically reactive metabolites. We need to be able to design out those particular functional groups that may lead to chemically reactive metabolite formation. We are going to use some of the *in vitro* systems that we have, and *in vivo* systems to be able to see what's going on at the sub-cellular level when

an individual or animal or test system is exposed to sub-therapeutic doses of drugs as well as supra-therapeutic doses of drugs, and see whether there is any kind of change in gene expression. Additionally, there are technologies available such as micro-arrays, that we may be able to utilise, but again, one is going to get a lot of data, and one needs to be able to make sense of that data. I think studies need to be done in those areas, in order to be able to see whether we can use molecular biology to find bio-markers which can then be used as surrogate end points, to predict the propensity of a drug to cause an adverse reaction.

**X. Carné:** From the epidemiological point of view, one of the most fascinating cases of severe type B reaction is the well-known agranulocytosis related to dipyrrone. I think it is a very interesting story, and probably you all know. In 1980, Herks performed an international study on the relationship between agranulocytosis and dipyrrone use. After more or less 10 years, several countries—Hungary, Italy, Spain, Germany, etc.—produced a big book explaining more or less that there were highly big differences on odd ratios in order of 20, between different countries. The paper was published, and it was highly criticised saying that they were strongly biased. In 1995, the Swedish government reintroduced dipyrrone in Sweden, and after 3 years they withdrew the drug again saying that the risk of this type B reaction was incredibly high for Swedish. We still use dipyrrone in Spain in a very high frequency, but I am deeply convinced that it is not a very big problem in Spain in practice; I feel that there are a lot of arguments to say that there are some genetic determinants in this relationship. There is a widely used drug all over the world and I have not read a single paper on this issue. Do you know something about it?

**M. Pirmohamed:** I agree it is an extremely interesting issue and we are just about to start working on it, actually. It is an interesting molecule and there are definite differences between different countries in terms of the relative incidences of agranulocytosis. If you look at the molecule, there is a suggestion that it may be bioactivated to a radical dication. However, that has not been proven; we need to be able to start off from the initial molecule, see how it is metabolised, whether it does form a reactive metabolite and then go on to look at further studies, patient samples, and so on, to be able to determine whether there is an immune basis to the agranulocytosis, and whether the individual predisposition lies within the immune site, for example in TNF or HLA. In fact, there is a paper from Bulgaria which actually looked at HLA types in patients who had agranulocytosis compared to those who did not have it but they only had four patients in the agranulocytosis group. They showed an HLA predisposition to dipyrrone and agranulocytosis. We are currently talking to Professor Laporte and trying to get some samples from Spain because dipyrrone is not licensed in the UK. We can actually look at it from an immunological point of view as well as, eventually, a genetic point of view.

**M. Reidenberg:** I would like to close this part of the morning session with a couple of comments. I will point out that genetics has been used for a long time in medicine, but then we called it family history and a couple of current examples have to do with how vigorously we try to get some asymptomatic person to have a colonoscopy depend on the frequency of colon cancer in first degree relatives of the patient. Family history is an official risk factor for when to start somebody with high cholesterol on a statin, or other cholesterol-lowering drug. The real difference that I see the new genetics is making is in the degree of precision and in the degree of detail. In order for us to handle, at the practice level, this degree of both precision in detail, it seems to me that we need a whole new

approach to information management. We need the information necessary at the time and place of need. There is no way that we can continue the traditional view that the physician must know all the facts. An example of dealing with this at our hospital was what to do with grapefruit juice and the issue of *CYP3A4*. We have the whole list of *CYP3A4* substrates, and there is no way that those of us at the bedside can possibly remember all the drugs on that list to remember the possible interaction of these drugs with grapefruit juice. As an administrative matter, we said there shall be no grapefruit juice in the New York Hospital. It was easy to do it through the diet kitchen. It was harder to implement with the contractors that filled the various juice and ice cream machines throughout the hospital for the visitors, and the concern that visitors would then bring somebody's favourite grapefruit juice to the bedside. The other issue that has not been raised in talking about this technology and its role in patient care is the responsibility of the health insurance industry in terms of both research and implementation, whether we are dealing with health insurance from the private or the public sector. Finally, I would like to thank both the speakers and the discussants for a most interesting morning session.

**M. Ingelman-Sundberg:** The highest number of registered deaths in Sweden is really a consequence of warfarin treatment, about 30–40 deaths a year. Probably, it is underestimated, so the real figure maybe 10 times higher actually. We had an interesting study by Ann Daly et al. from Newcastle, where the results indicated that at least the *CYP2C9* genotyping could help in predicting bleeding complications. You also presented another study, performed by Taube et al., which essentially showed very little effect of the genotype on the outcome; I would like to ask you, when you compare these two papers, what are the main differences? What is the cause for these quite opposite findings in a very important field?

**M. Pirmohamed:** Ann Daly et al. have gone into a clinical population and have selected those patients who were on 1.5 mg less and then selected a random group; and they only looked at a proportion of patients. If you look at Taube's study, which is a much larger study, what they did was to take the whole clinic population; in that clinic, what they were actually doing was using computers to be able to do the prescribing. In a way, computer was important there in terms of improving the prescribing. One does need to take into account confounding factors, and genotype may be important in improving prescribing of warfarin in the future, but I think that it needs to be studied in a prospective fashion, but may be combining computer and genotyping may be the answer for the future, but it really does need a prospective study. In summary, it was patient selection that was the most important criteria in terms of determining the difference between the two studies.

**G. Levy:** In the case of warfarin, as I have shown on the last slide in my presentation, simply focussing on INR and appropriate dose modulation to stay within the therapeutic range, and focussing on lifestyle and on diet, is enough to make about a threefold difference in terms of either the incidence of thromboembolism on the one extreme or a serious or fatal bleeding. I think it very much is a matter of using feedback information, if you will and in many cases, feedback information, be it concentration control or some other criteria, will do a great deal already now. We have the tools to accomplish much more than is being done now in dose optimisation. Hopefully, genotyping or phenotyping will do even better, but I am reminded of the very limited use of phenotyping with respect

to acetylation of isoniazid. It really is not widely used now, whether the pay-off was not there, or whether people just did not care, but certainly today there are many tools that are not used at the optimum.

**M. Reindenberg:** I also think that, at least in science, there are two different questions: one is, does a particular factor influence dose response? The other one is, how much of the variation that we see in practice is due to that factor? Our own work with warfarin would illustrate it; there was a British study asking if age is an important factor modifying warfarin dose response. There were four healthy young people and four healthy elderly people in the study, and age made a difference; the old people had a greater intensity of effect. Another study from an ambulatory anti-coagulant clinic, looking at dose response showed that while age had an effect, it only accounted for 8% of the variance in the total population. We were interested in the issue in in-patients starting anti-coagulation, and our study was done to see if we could determine, based on an index of the first couple of days of treatment, whether that could predict chronic therapy. What we found in our population of around 60 consecutive inpatients was that age made no difference whatsoever in dose response and the reason was that patients were taking multiple drugs and had confounding diseases. About 5 out of 60 people were on phenobarbital, and equal number had elevated bilirubin. The point that I am trying to make is that the question “does a factor influence dose response?” is very different from the question “how much of the variance in practice is due to that factor?”

**L. Sheiner:** The percent variance explained is a design-dependent estimand; not a biological one. If you have no variation in renal function in the population that is given a drug that is excreted 100% renally, you will have 0% of variance explained by renal function.

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