Genetic susceptibility to adverse drug reactions

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Adverse drug reactions (ADRs) are a major clinical problem. Genetic factors can determine individual susceptibility to both dose-dependent and dose-independent ADRs. Determinants of susceptibility include kinetic factors, such as gene polymorphisms in cytochrome P450 enzymes, and dynamic factors, such as polymorphisms in drug targets. The relative importance of these factors will depend on the nature of the ADR; however, it is likely that more than one gene will be involved in most instances. In the future, whole genome single nucleotide polymorphism (SNP) profiling might allow an unbiased method of determining genetic predisposing factors for ADRs, but might be limited by the lack of adequate numbers of patient samples. The overall clinical utility of genotyping in preventing ADRs needs to be proven by the use of prospective randomized controlled clinical trials.

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Adverse drug reactions (ADRs) remain a major clinical problem. A recent meta-analysis suggested that in the USA in 1994, ADRs were responsible for >100 000 deaths, making them between the fourth and sixth commonest cause of death[1]. Although these figures have been heavily criticized[2], they emphasize the importance of ADRs. Indeed, there is good evidence that ADRs account for 5% of all hospital admissions[3] and increase the length of stay in hospital by two days at an increased cost of ~$2500 per patient[4,5]. ADRs are also one of the commonest causes of drug withdrawal[6], which has enormous financial implications for the pharmaceutical industry.

ADRs, perhaps fortunately, only affect a minority of those taking a particular drug. Although factors that determine susceptibility are unclear in most cases, there is increasing interest in the role of genetic factors. Indeed, the role of inheritable variations in predisposing patients to ADRs has been appreciated since the late 1950s and early 1960s through the discovery of deficiencies in enzymes such as pseudocholinesterase (butyrylcholinesterase) and glucose-6-phosphate dehydrogenase (G6PD). More recently, with the first draft of the human genome just completed, there has been renewed interest in this area with the introduction of terms such as pharmacogenomics and toxicogenomics[7]. Essentially, the aim of pharmacogenomics is to produce personalized medicines, whereby administration of the drug class and dosage is tailored to an individual genotype[8]. Thus, the term pharmacogenomics embraces both efficacy and toxicity. Although the lack of a therapeutic effect of a drug can be regarded as an unwanted effect, this article will focus on true ADRs, which have recently been defined as 'an appreciably harmful or unpleasant reaction, resulting from an intervention related to the use of a medicinal product, which predicts hazard from future administration and warrants prevention or specific treatment, or alteration of the dosage regimen, or withdrawal of the product.'[9] Numerous examples of genetic predisposition to ADRs exist in the literature; however, in this article, specific areas will be emphasized to illustrate concepts, highlight potential problems and determine future promise.

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 might be important	Dependent on (usually uncharacterized) 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
Overall proportion of adverse drug reactions	80%	20%
First detection	Phases I–III	Usually Phase IV, occasionally Phase III
Animal models	Usually reproducible in animals	No known animal models

Classification of adverse drug reactions

From a clinical perspective, ADRs can be divided into two broad types, type A and type B (Ref. 10) (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. By contrast, type B ADRs are bizarre reactions that are unpredictable from the known pharmacology of the drug and show no apparent dose–response relationship[11]. Typically, type A ADRs have been labelled as host independent (i.e. not dependent on genetic factors). Clearly, this is an over-simplification because there is now increasing evidence for a role for genetics in the determination of drug disposition and drug response and, thus, susceptibility to ADRs. In general, genetic predisposition to a type A ADR will conform to a monogenic or oligogenic model whereas type B ADRs might be similar to complex human diseases in having a polygenic predisposition[12]. This might make

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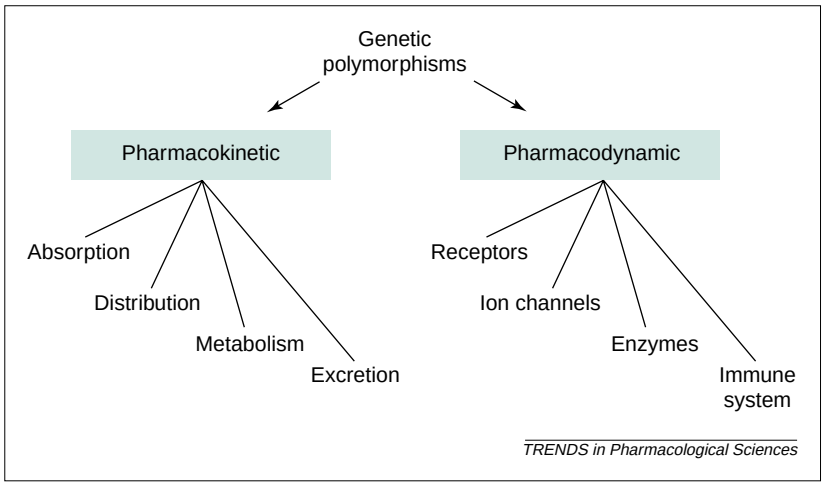

Fig. 1. Genetic variability leading to susceptibility to adverse drug reactions can affect both pharmacokinetic and pharmacodynamic pathways.

detection and prevention of type B ADRs more difficult than for type A ADRs.

Genetic polymorphisms and adverse drug reactions

A gene can be defined as exhibiting a genetic polymorphism if the variant allele exists in the normal population at a frequency of at least 1% (Ref. 13). Genetic polymorphisms are a source of variation of drug response in the human body. In relation to ADRs, most interest has centred on the involvement of pharmacokinetic factors and, in particular, drug metabolism. However, there is now increasing realization that genetic variation in drug targets (pharmacodynamic factors) might also predispose to ADRs, although research into this area is in its infancy (Fig. 1). It is important to note that although the focus of this review is genetic sources of variation, environmental factors such as disease, alcohol, smoking and diet might also be significant sources of variability and might predominate. Indeed, the environment might interact with the genetic factors and either increase or decrease the risk of an ADR.

Table 2. Cytochrome P450 enzyme gene polymorphisms and putative adverse drug reactions

P450 enzyme	Drug[a]	Adverse reaction	Refs
CYP1A2	Typical antipsychotics	Tardive dyskinesia	14
CYP2C9	Warfarin	Haemorrhage	
	Tolbutamide	Hypoglycaemia	13,15
	Phenytoin	Phenytoin toxicity	
CYP2C19	Mephenytoin	Neurotoxicity	13
	Diazepam	Prolonged sedation	
CYP2D6	Antiarrhythmics	Arrhythmias	13,16,17
	β-Blockers	Bradycardia	
	Tricyclic antidepressant	Confusion	
	Opioids	Dependence	
	Phenformin	Lactic acidosis	
	Perhexilene	Hepatotoxicity	
CYP3A4	Anti-leukaemic agents	Treatment-related leukaemia	18

[a]The list of drugs for each P450 enzyme is not exhaustive, with few relevant examples chosen.

Phase I metabolic pathways

Several polymorphisms that affect genes encoding cytochrome P450 enzymes have been described (Table 2), although polymorphisms in the gene encoding CYP2D6 have attracted most attention. This P450 isoform is responsible for the metabolism of ~25% of drugs, including certain antidepressants, antipsychotics, β-blockers and opioid analgesics. Variability in the rate of drug metabolism by CYP2D6 is >100-fold; this is genetically determined with 6% of the caucasian population carrying two null alleles at the *CYP2D6* gene locus[19]. There are >70 variant alleles of the *CYP2D6* locus, with 15, including the two most common variants *CYP2D6*4* and *CYP2D6*5*, encoding non-functional products. Other variants that decrease activity (e.g. *CYP2D6*10*), alter substrate specificity (e.g. *CYP2D6*17*) or increase activity (e.g. *CYP2D6*2xN*) have also been described[20]. Absent or reduced CYP2D6 activity can lead to ADRs by the following mechanisms[16]: (1) decreased first pass metabolism and drug elimination (e.g. metoprolol and bradycardia); (2) accumulation of the drug as a result of reduced metabolism (e.g. perhexilene and hepatotoxicity); and (3) re-routing of metabolism (e.g. phenacetin and methaemoglobinaemia). However, despite evidence that the *CYP2D6* polymorphism affects the response to therapeutic agents, it is not routine clinical practice to prospectively type patients before starting a course of treatment using a drug that is metabolized by CYP2D6 (Ref. 21). Indeed, of the many drugs that are metabolized by CYP2D6, only perhexilene, which was associated with a higher risk of hepatotoxicity and neuropathy in CYP2D6 poor metabolizers, has been withdrawn. Possible reasons for why CYP2D6 typing is not routinely performed in clinical practice include the fact that: (1) phenotyping and genotyping procedures are not routinely available; (2) typing procedures are expensive; (3) many of the drugs metabolized by CYP2D6 have a wide therapeutic index and adverse effects suffered are mild, and these are amenable to dose reduction; and (4) routine typing has not been shown to be cost effective. Prospective randomized controlled trials are needed to show that CYP2D6 typing is both clinically effective and cost effective. A recent pilot study has suggested that the CYP2D6 poor metabolizers treated with psychotropic agents might be at higher risk of ADRs, have a prolonged hospital stay and higher treatment costs[22]. However, this study was only performed in 100 patients and thus the study needs to be replicated using a larger number of patients.

The role of genetic variation in the metabolism of warfarin by CYP2C9 has attracted a great deal of attention recently. Warfarin is the oral anticoagulant of choice in the UK for the treatment of venous thromboembolism, and prophylaxis against

Table 3. Phase II drug metabolizing enzyme gene polymorphisms and putative adverse drug reactions

Phase II enzyme	Drug	Adverse reaction[a]	Refs
Plasma butyrylcholinesterase	Succinylcholine	Prolonged apnoea	35
N-acetyltransferase	Sulfonamides	Hypersensitivity	36
	Amonafide	Myelotoxicity	
	Procainamide, hydralazine, isoniazid	SLE	
Thiopurine methyltransferase	6-Mercaptopurine, azathioprine	Myelotoxicity, treatment-related second tumours	37
Dihydropyrimidine dehydrogenase	5-Fluorouracil	Myelotoxicity	38
UDP glucuronosyl transferase 1A1	Irinotecan	Diarrhoea, myelosuppresion	39

[a]Abbreviation: SLE, systemic lupus erythematosus.

thromboembolism in patients with valvular heart disease and atrial fibrillation[23]. The number of patients attending anticoagulant clinics has doubled in the past five years and the trend is set to continue. The major risk of warfarin treatment is haemorrhage, with between eight and 26 patients having a bleed for every 100 patients treated with warfarin for a year[24]. The risk of bleeding increases with the intensity of anticoagulation in a log linear fashion. Minimization of the risk of bleeding (i.e. an improvement in the benefit–risk ratio) depends on accurate prediction of the dosage requirements during warfarin therapy. However, this is difficult because there is a wide interindividual variability in the dose necessary to maintain the international normalized ratio [INR (a biomarker of the degree of anticoagulation)] within a target range. Furthermore, the process used to arrive at the final dose depends on clinical judgement and thus bleeding associated with warfarin remains a common clinical problem.

The *S*-enantiomer of warfarin, which is predominantly responsible for the anticoagulant effect, is metabolized by CYP2C9 (Ref. 25). Polymorphisms in the *CYP2C9* gene result in at least two allelic variants, *CYP2C9*2*, where cysteine substitutes for arginine at position 144, and *CYP2C9*3*, where leucine substitutes for isoleucine at residue 359 in the substrate binding site[26]. Decreased clearance of warfarin by the allelic variants has been shown *in vitro*[27,28] and *in vivo*[28]. 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[15]. Although the relationship between *CYP2C9* genotype and dose requirement has been confirmed in two other studies[29,30], one of which was much larger (n = 561)[29], the relationship with severe over-anticoagulation and hence bleeding was not.

On the basis of these studies, should all patients who are beginning a course of warfarin treatment be genotyped? This is probably premature because several confounding factors need to be studied. First, the anticoagulant response is partly dependent on *R*-warfarin, which is metabolized by CYP1A2 and CYP3A4 (Ref. 31). Second, there are several pharmacodynamic factors, such as vitamin K status and thyroid disease, that alter sensitivity to anticoagulants[32]. Third, there are mutations in clotting factors such as prothrombin that might alter sensitivity to warfarin[29]. Fourth, there are other methods of dose titration and dose maintenance with warfarin (e.g. prescribing by computer program[33] or home monitoring[34]) that 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 hours of admission of patients to hospital. Thus, before genotyping prior to warfarin treatment can become a routine part of clinical practice, there is a need for a prospective randomized clinical trial that not only incorporates into its trial design the different methods for monitoring and altering warfarin dosage, but also the confounding factors mentioned above.

Phase II metabolic pathways

Many Phase II drug metabolizing enzymes have been shown to be polymorphically expressed (Table 3), which in turn has led to the occurrence of ADRs with several drugs. *N*-acetyltransferase type 2 (NAT-2) was one of the first Phase II enzymes discovered to be polymorphically expressed[36]. However, largely for the same reasons outlined for CYP2D6 above, patients are not routinely typed for NAT-2 before starting drug therapy. The glutathione-*S*-transferase enzyme gene polymorphisms, although of interest with respect to predisposition to cancer, have largely proved negative in studies examining their role in idiosyncratic toxicity with several drugs including sulfamethoxazole[40], anticonvulsants[41] and tacrine[42,43]. Perhaps the greatest impact with respect to polymorphisms in Phase II pathways in the future will be in the field of oncology. Two examples, thiopurine methyltransferase (TPMT) and UDP-glucuronosyl transferase (UGT) 1A1 gene polymorphisms, are discussed in detail below.

TPMT

TPMT is involved in the metabolism of 6-mercaptopurine (6-MP) and its pro-drug azathioprine[44], which both require metabolism to thioguanine nucleotides to exert cytotoxicity[37]. TPMT activity reduces the formation of thioguanine nucleotides whereas cellular accumulation of thioguanines is inversely related to TPMT activity. 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[45], the most common alleles being *TPMT*2*, *TPMT*3A* and *TPMT*3C*. At least 10% of caucasians exhibit intermediate activity (i.e. are heterozygotes)

Table 4. Pharmacogenetic defects in enzymes that lead to undesirable pharmacodynamic adverse effects[a]

Enzyme defect	Drug	Adverse reaction
Glucose-6-phosphate dehydrogenase deficiency	Primaquine, sulfonamides, dapsone, nitrofurantoin	Haemolytic anaemia
Methaemoglobin reductase deficiency	Nitrites, dapsone	Methaemoglobinaemia, haemolysis
Porphobilinogen deaminase deficiency (acute intermittent porphyria)	Barbiturates, estrogens, alcohol, anticonvulsants and sulfonamides	Acute porphyric crises
Acetylcholinesterase	Anticholinesterase agents	Neurotoxicity

[a]Adapted from Ref. 59.

whereas 1 in 300 have low or no detectable TPMT activity[45]. A good correlation exists between the presence of *TPMT*2*, *TPMT*3A* and *TPMT*3C* alleles and phenotype[46]. Patients with deficient TPMT activity can develop fatal haemopoietic toxicity with full doses of 6-MP, whereas a reduction in the dosage by 90–94% can lead to successful treatment without such toxicity[47,48]. By contrast, patients with wild-type alleles might require higher dosages to ensure efficacy in the treatment of acute lymphoblastic leukaemia[49]. Heterozygous patients tolerate azathioprine and 6-MP for shorter periods than patients with wild-type alleles. Furthermore, dosage reduction for the prevention of toxicity was required for 76% of the treatment time in patients who were homozygous for the variant *TPMT* alleles[50]. Such reduction in toxicity by dose reduction must be counterbalanced by the fact that a high dose intensity of 6-MP is an important predictor of event-free survival in childhood leukaemia[51]. Children with TPMT deficiency also appear to be at higher risk of secondary myelodysplasia or acute myeloid leukaemia, possibly as a result of DNA damage through accumulation of thioguanine nucleotides[37,52]. A biochemical assay of erythrocyte lysates is currently used to assess TPMT activity[45,53]; however, spurious results can be obtained when patients have been given blood transfusions, a frequent occurrence in this group of patients. Genotyping for *TPMT*2*, *TPMT*3A* and *TPMT*3C* can predict TPMT status in 80–95% of patients[37]. DNA chip technology offers the opportunity of being able to detect all known inactivating mutations with almost complete predictive power[45]. Thus, TPMT is a clear example of a clinically significant genetic polymorphism where prospective genotyping might allow individualization of drug therapy and thereby maximize efficacy and minimize toxicity.

UGT

The UGTs are a superfamily of Phase II drug metabolizing enzymes that can be divided into two major classes, UGT1 and UGT2. UGT1 is important for the glucuronidation of bilirubin; decreased activity of this enzyme has been reported in patients with Crigler–Najjar syndrome or Gilbert's syndrome[54]. Irinotecan is a camptothecin analogue that has strong anti-tumour activity. However, its use can be limited because of the occurrence of severe dose-dependent adverse reactions such as diarrhoea and leucopaenia. Irinotecan is a pro-drug and is metabolized by carboxylesterase to form the active SN-38, which undergoes glucuronidation by UGT1A1; there is a correlation between the *UGT1A1* promoter region polymorphism and phenotype[55,56]. Furthermore, a recent study in Japanese patients has shown that a heterozygous or homozygous genotype for *UGT1A1*28* in the promoter region was a significant risk factor for irinotecan toxicity[39]. No association was observed with coding region polymorphisms. However, not all patients with the variant *UGT1A1*28* genotype experienced toxicity, and thus other factors such as body surface area and concomitant therapy will also be important in the determination of toxicity. A prospective trial to elucidate the usefulness of genotyping before irinotecan therapy is warranted on the basis of these initial findings.

Transporters

Membrane transporters play an important role in the absorption, distribution and excretion of drugs. A large number of transporters have now been described, which should be a fruitful area for future research into the causes of variability in drug response. To date, most studies have focused on the multi-drug resistance gene (*ABCB1*), which encodes P-glycoprotein (Pgp), an efflux pump with wide substrate specificity that has been implicated in resistance to anti-tumour agents[57]. A recent study identified a functionally active polymorphism in exon 26 of the *ABCB1* gene; individuals homozygous for this polymorphism (*T/T* genotype) had the lowest Pgp expression and the highest digoxin plasma concentrations compared with individuals with the wild-type *C/C* phenotype[58]. Whether this polymorphism increases the risk of toxicity associated with digoxin and with other Pgp substrates, such as protease inhibitors and cyclosporin, requires further study.

Enzymes

G6PD

Genetically determined variation in enzyme structure might render the enzyme more sensitive to the action of a drug, resulting in toxicity (Table 4). The archetypal example is G6PD deficiency, a sex-linked disorder that affects ~200 million people worldwide[60]. The incidence and severity of the enzyme deficiency vary with race, which reflects the large number (~400) of variants that have been described. In the majority of individuals, the deficiency causes haemolysis only in the presence of stress (e.g. infection or drugs). A large number of drugs have been reported to induce red cell haemolysis in

patients with a deficiency of G6PD. Normally, G6PD functions to reduce NADP while oxidizing glucose-6-phosphate, thus providing a source of reducing power that maintains cellular glutathione in the reduced form. In the absence of reduced glutathione, the red cell is susceptible to oxidative damage from drugs, which is manifest clinically as haemolysis, a fall in the concentration of haemoglobin, fever and the formation of dark urine.

Acetylcholinesterase

A promoter polymorphism that impairs the transcriptional response of acetylcholinesterase on exposure to inhibitors of this enzyme has recently been implicated as a novel mechanism for the neurotoxic adverse effects occasionally observed with anticholinesterases[61], which are increasingly being used in the treatment of Alzheimer's disease. Two functionally active variants with allele frequencies of 0.006 and 0.012 were identified. A patient who showed increased sensitivity to the acetylcholinesterase pyridostigmine was found to have increased constitutive concentrations of acetylcholinesterase as a result of disruption of two adjacent HNF3 (hepatocyte nuclear factor 3) binding sites. The increased sensitivity was postulated to be due to impairment in the production of fresh (and therefore uninhibited) enzyme on exposure to the anticholinesterase. Interestingly, the authors also postulated that these promoter polymorphisms might be of importance in multiple chemical sensitivity syndrome, a poorly defined syndrome with an unclear pathogenesis.

Receptors

The therapeutic response to a drug acting on a receptor might be modulated by genetic variation in that receptor as has been shown for β_2-adrenoceptor agonists[62] and antipsychotics such as clozapine[63]. However, such variation might also increase the sensitivity of that receptor and lead to adverse effects. For example, a serine to glycine substitution in the gene encoding the dopamine D3 receptor has recently been shown to predispose to the occurrence of tardive dyskinesia following treatment with typical neuroleptic agents[64]. Alteration of the tertiary structure of the D3 receptor leading to a higher affinity for dopamine might be the mechanistic basis of this association and is in accordance with the hypothesis that tardive dyskinesia is due to a supersensitivity to dopamine. Receptor variation might also lead to unpredictable toxicities, as observed in the development of malignant hyperthermia following administration of general anaesthetics such as halothane[65]. Mutations in the gene encoding the ryanodine receptor are thought to account for susceptibility to malignant hyperthermia in >50% of cases. The defect in the ryanodine receptor makes this receptor more sensitive to lower concentrations of stimulators of opening, resulting in enhanced rates of Ca^{2+} release from the sarcoplasmic reticulum during anaesthesia; this, in turn, leads to the sustained muscle contraction and glycolytic and anaerobic metabolism that is characteristic of malignant hyperthermia. Pre-anaesthetic prediction of susceptibility can be undertaken in patients with a family history, using an invasive phenotypic test (caffeine–halothane contracture test)[65]. The genetic heterogeneity (at least 22 missense mutations have been identified) together with variable penetrance and unidentified defects in 50% of patients means that genetic testing for diagnosing susceptibility is currently not acceptable.

Ion channels

Recently, the use of several drugs including terfenadine, cisapride, thioridazine and sertindole has been restricted because of the occurrence of QT-interval prolongation on the electrocardiogram (ECG) and occasionally torsades de pointes (TdP)[66]. Mutations in various ion channels that are responsible for normal ventricular repolarization have been reported in patients with inherited long-QT syndromes[67]. All drugs that are known to cause prolongation of the QT interval preferentially block the rapid delayed rectifier K^+ current [$I_{K(Vr)}$]. It has therefore been hypothesized that genetically determined variation might determine individual predisposition to drug-induced QT prolongation and, indeed, studies appear to support this hypothesis. For example, a patient with clarithromycin-induced TdP carried a missense mutation in *KCNE2;* this altered the K^+channel such that the affected subunit MinK-related peptide 1 activated less readily at baseline and was threefold more sensitive to drug inhibition than the wild-type channel[68]. A further study examining *KCNE2* in 98 unrelated individuals has identified a single nucleotide polymorphism (SNP) present in 1.6% of the population[69]. The presence of this SNP in one patient was associated with the development of a prolonged QT interval following administration of sulfamethoxazole-trimethoprim, possibly by accelerating the deactivation of the channel. To date, these studies have been performed in a small number of individuals. Larger-scale studies in patients with an accurate clinical and electrocardiographic characterization of the degreee of QT prolongation in response to different drugs will be necessary to examine the utility of SNP screening of genes encoding $K_{V(r)}$ subunits as a means of predicting susceptibility to, and therefore preventing, QT-interval prolongation and TdP.

Immune response genes

Approximately 20% of all ADRs are thought to have an immunological aetiology[70], and are categorized as type B ADRs (Table 1; although it is important to remember that not all type B reactions are immune mediated). Bioactivation of drugs to chemically reactive intermediates that act as haptens is

Table 5. HLA and adverse drug reactions[a,b]

Drug	Adverse reaction	HLA association
Carbamazepine	Severe hypersensitivity reactions	DR3, DQ2
Clozapine	Agranulocytosis	B38, DR4, DQ3
Dipyrone	Agranulocytosis	A24, B7, DQ1
Gold	Proteinuria, dermatological reactions, thrombocytopenia	DR3
Hydralazine	SLE	DR4
Levamisole	Agranulocytosis	B27
Oxicam	Toxic epidermal necrolysis	A2, B12
Penicillamine	Penicillamine toxicity	DR3
Sulfonamides	Toxic epidermal necrolysis	A29, B12, DR7

[a]The list of HLA associations is not exhaustive. Importantly, most of these findings are based on single studies, and therefore do need replication. Table is adapted from Ref. 35.
[b]Abbreviations: HLA, human leukocyte antigen; SLE, systemic lupus erythematosus.

important in the pathogenesis of these ADRs (Ref. 11). Predisposition to such ADRs is thought to be multifactorial, involving many genes that interact with environmental factors[12]. In a similar fashion to complex diseases[71], it is likely that there will be heterogeneity in that different combinations of gene variants give rise to a similar phenotype. Furthermore, preliminary evidence indicates that the frequency of a polymorphism contributing to the phenotype will be only slightly elevated in the disease group when compared with unaffected controls. Thus, prevention of these ADRs will be difficult and will require collection of DNA samples from well-characterized cohorts of patients with defined toxicities.

Initial investigations into the genetic predisposing factors for immune-mediated ADRs largely focused on the role of the major histocompatibility complex (MHC)[35]. HLA (human leukocyte antigen) phenotyping and, latterly, genotyping has identified various HLA types that are associated with ADRs (Table 5). However, given that the MHC is located on the short arm of chromosome 6, which is a hot spot for polymorphisms, and that 60% of genes in that region are of unknown function[72], it is not entirely clear whether the different HLA types are true predisposing factors or are in linkage disequilibrium to more relevant genes nearby. This issue will be a major challenge of research in this area.

Also located within the MHC in the class III region is the tumour necrosis factor α (TNF-α) locus[73]. TNF-α is a pro-inflammatory cytokine, over-secretion of which might contribute to the tissue damage observed in immune-mediated hypersensitivity reactions. We have recently investigated this in relation to carbamazepine (CBZ) hypersensitivity, which has a complex pathogenesis[74]. CBZ hypersensitivity syndrome is characterized by skin rash, fever, eosinophilia, lymphadenopathy and extra-cutaneous manifestations. Skin biopsy data have demonstrated the involvement of cytotoxic T cells and pro-inflammatory cytokines such as TNF-α (Ref. 75). There is both clinical and biochemical data that suggest that this form of idiosyncratic toxicity has a genetic basis[76,77]. *Ex vivo* studies have shown that cells from hypersensitive patients are more susceptible to the toxic effects of drug metabolite(s) generated *in situ*[78,79]. However, analysis of genes encoding enzymes that are responsible for drug bioinactivation, including microsomal epoxide hydrolase, glutathione transferases, catechol-*O*-methyl transferase and quinone reductase, failed to reveal an association with CBZ hypersensitivity[41,80,81]. Analysis of the promoter region polymorphisms in the gene encoding TNF-α that might be functionally active has shown that serious, but interestingly not non-serious, hypersensitivity reactions to CBZ showed an association with the –308, but not the –238, polymorphism[82]. Although demonstration in an independent sample population is required to confirm this association, we have shown a biochemical rationale for TNF-α in the pathogenesis of the hypersensitivity reactions and have thus satisfied two out of the three criteria proposed by Todd[83] to define a relationship between a clinical phenotype and a SNP.

Future perspectives and conclusions

The current approach to the identification of 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 to use a comprehensive, densely spaced, genome-wide SNP map, which might allow us in the future to conduct screens for pharmacogenetically active genes as whole-genome, unbiased searches[84]. SNPs are single-base differences in the DNA sequence, observed between individuals, which occur throughout the human genome. The International SNP Map Working Group has recently published a map of 1.42 million SNPs throughout the genome, occurring at an average density of one SNP every 1.9 kilobases[85]. At least 60 000 SNPs are within coding regions (coding SNPs) and are therefore more likely to be functionally active. This high-density SNP map provides an opportunity to perform SNP profiling to identify genetic factors predisposing to ADRs. However, many obstacles remain before SMART cards[86] carrying SNP pharmacogenetic profiles become a reality; some of these include: (1) the development of technologies to perform SNP profiling; (2) the development of less expensive genotyping strategies; and (3) consideration of ethical requirements to ensure that this does not result in a genetic underclass that finds it difficult, if not impossible, to obtain personal and healthcare insurance. Furthermore, 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 reported that for testing 100 000 loci in a genome-wide screen will require a threefold greater

sample size at a significance level of 2.5×10^{-7} (Ref. 87). This suggests 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[21].

Therefore, there is increasing evidence to implicate genetic factors in predisposing to ADRs. Investigation to date has relied on the candidate gene approach and has focused on drug metabolizing enzymes. There is now a need to widen the search for candidate genes to include more pharmacodynamic targets, which, in the case of immune-mediated ADRs, should also focus on downstream events that are responsible for determining immune responsiveness and tissue injury. The availability in the near future of cost-effective SNP genotyping technologies might allow an unbiased search for pharmacogenetically active genes that are important in predisposition to ADRs. However, this approach is likely to be limited by the lack of availability of suitable patient samples, particularly for the rare ADRs, unless steps are taken to set up multi-centre international collaborations. Even when one gene is responsible for determining susceptibility to an ADR, it is important to examine both the clinical- and cost-effectiveness of prospective genotyping in randomized controlled trials.

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