Pharmacogenetics and human genetic polymorphisms

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The term pharmacogenetics was first used in the late 1950s and can be defined as the study of genetic factors affecting drug response. Prior to formal use of this term, there was already clinical data available in relation to variable patient responses to the drugs isoniazid, primapine and succinylcholine. The subject area developed rapidly, particularly with regard to genetic factors affecting drug disposition. There is now comprehensive understanding of the molecular basis for variable drug metabolism by the cytochromes P450 and also for variable glucuronidation, acetylation and methylation of certain drugs. Some of this knowledge has already been translated to the clinic. The molecular basis of variation in drug targets, such as receptors and enzymes, is generally less well understood, although there is consistent evidence that polymorphisms in the genes encoding the β-adrenergic receptors and the enzyme vitamin K epoxide reductase is of clinical importance. The genetic basis of rare idiosyncratic adverse drug reactions had also been examined. Susceptibility to reactions affecting skin and liver appears to be determined in part by the HLA (human leucocyte antigen) genotype, whereas reactions affecting the heart and muscle may be determined by polymorphisms in genes encoding ion channels and transporters respectively. Genome-wide association studies are increasingly being used to study drug response and susceptibility to adverse drug reactions, resulting in identification of some novel pharmacogenetic associations.

Key words: adverse drug reaction (ADR), cytochrome P450, genome-wide association study, human gene polymorphism, human leucocyte antigen (HLA), pharmacogenetics.

INTRODUCTION
Pharmacogenetics can be defined as the study of genetic factors affecting drug response. There is considerable overlap between pharmacogenetics and the much newer discipline of pharmacogenomics. Pharmacogenomics is sometimes described as the whole-genome application of pharmacogenetics, which has traditionally been considered to be concerned with single-gene effects. Pharmacogenomics may also extend to the development of new drugs using genomic information. The terms pharmacogenetics and pharmacogenomics are often used interchangeably. In the present review article, the term pharmacogenetics is used throughout and encompasses both genetic and genomic examples.

The genetic basis for inter-individual variation in drug response has been studied extensively over the last 50 years. It is now recognized that all human genes are subject to extensive genetic polymorphism, with many of these polymorphisms resulting in functionally significant effects. A genetic polymorphism is defined as the occurrence, together in the same population, of more than one allele or genetic marker at the same locus with the least frequent allele or marker occurring more frequently than can be accounted for by mutation alone. Generally the less frequent marker needs to occur at a population frequency of at least 1% for the variation to be considered a true genetic polymorphism, with less frequent variants often classed as isolated mutations. Although many polymorphisms don’t have functionally significant effects, those that result in either altered expression or activity of the gene product are those usually studied in pharmacogenetic studies. Most current pharmacogenetic studies involve study of drug response in unrelated individuals rather than in families. This means that most genetic variants examined are seen at the frequency of 1% or higher, typical of a polymorphism, rather than the focus being on rare variants, as would be the case in more traditional genetic studies on rarer single-gene disorders.

The HapMap project (http://hapmap.ncbi.nlm.nih.gov/), and other approaches, such as genome-wide association studies, have allowed the identification of a number of novel genetic factors affecting disease susceptibility. These developments have also been important in the area of pharmacogenetics although much of our present detailed knowledge on polymorphisms affecting drug response predates these developments. At least in part, this is due to the fact that methods used to study individual phenotypes in relation to drug response have been available for many years and this enabled specific polymorphisms correlating with phenotypes to be identified during the 1980s and 1990s when gene cloning became widely available.

The present review article is concerned particularly with recent developments in the area of pharmacogenetics but will also consider historical aspects and review the well-established knowledge in areas such as the pharmacogenetics of drug metabolism. The last 5 years has seen considerable progress in understanding the pharmacogenetics of drug transporters and various drug targets, the basis of certain idiosyncratic ADRs (adverse drug reactions) and in the use of genome-wide association studies to study drug response. These areas will be discussed in detail together with some important recent developments in the cytochrome P450 field, such as establishment

Abbreviations used: ABC, ATP-binding-cassette; ACE, angiotensin-converting enzyme; ADR, adverse drug reaction; CBZ, carbamazepine; DILI, drug-induced liver injury; GSTM1, glutathione transferase mu 1; HLA, human leucocyte antigen; HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; 5-HT, 5-hydroxytryptamine; NAT2, N-acetyltransferase 2; OCT, organic cation transporter; PXR, pregnane X receptor; SJS, Stevens–Johnson syndrome; SLC, solute carrier; SLCO, solute carrier organic anion; SNP, single nucleotide polymorphism; TPMT, thiopurine S-methyltransferase; UGT, UDP-glucuronosyltransferase; VKORC1, vitamin K epoxide reductase complex 1.

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of a major role for the CYP2D6 genotype in predicting response to tamoxifen and for CYP2C19 with respect to clopidogrel response.

HISTORICAL ASPECTS OF PHARMACOGENETICS

The foundation for much of modern pharmacogenetics came from experiments on chemical metabolism during the 19th century. Among the findings from those studies were that benzoic acid undergoes conjugation with glycine in vivo in both humans and animals, that some compounds can undergo conjugation with acetate and that benzene is oxidized to phenol in both dogs and humans (for a review, see [1]).

Observations by Garrod on the possibility of variation in chemical metabolism in the early 20th century have been well-reviewed [2]. The first direct pharmacogenetic study was reported in 1932 when Snyder [3] reported on the ability to taste phenylthiocarbamide within families and showed that this trait was genetically determined. The gene responsible for this variation, and its common genetic polymorphisms, have only been identified more recently (for a perspective, see [4]). This gene, TAS2R38 (taste receptor, type 2, member 38) encodes a small protein found at the apical membrane of taste-receptor cells. Non-tasters of phenylthiocarbamide differ from tasters by three different amino acids [4]. Phenylthiocarbamide shows similarity to prescribed drugs, such as propylthiouracil, but whether this is of relevance in the response to these drugs is not clear.

During the 1950s, reports on drug-specific pharmacogenetic observations concerned with three widely used drugs, isoniazid, primaquine and succinylcholine, appeared. The earliest report concerned primaquine whose use was found to be associated with acute haemolysis in a small number of individuals [5]. Subsequent studies showed that this toxicity was due to absence of the enzyme glucose-6-phosphate dehydrogenase in red blood cells of affected individuals [6]. The molecular genetic basis of this deficiency was later established by Hirano and Beutler [7] in 1988.

Isoniazid was first used to treat tuberculosis in the early 1950s. As reviewed recently, its use represented an important advance in the treatment of tuberculosis [8]. Variation between individuals in urinary excretion profiles was described by Hughes [9] and shortly afterwards an association between the metabolic profile and the incidence of a common adverse reaction, peripheral neuritis, was reported, with those showing slow conversion of the parent drug into acetylisoniazid more susceptible [10]. Further studies [11–13] led to the conclusion that isoniazid acetylation was subject to a genetic polymorphism with some individuals (approx. 10% of East Asians, but 50% of Europeans) being slow acetylators. Slow acetylation was shown to be a recessive trait. As summarized below, the biochemical and genetic basis of slow acetylation is now well understood.

Also during the 1950s, a rare adverse response to the muscle relaxant succinylcholine was found to be due to an inherited deficiency in the enzyme cholinesterase [14]. Succinylcholine is used as a muscle relaxant during surgery and those with the deficiency show prolonged paralysis (succinylcholine apnoea). This observation was then extended by Werner Kalow who showed that the deficiency is inherited as an autosomal recessive trait and developed a biochemical test to screen for the deficiency (see [15] for his own description of that work). The enzyme encoded by the gene involved, which is now usually referred to as butryrylcholinesterase, has been well studied and a number of different mutations responsible for the deficiency have been identified. However, the original biochemical test is still the preferred method for identifying those affected by succinylcholine apnoea due to both the rarity of the problem and the range of different causative mutations.

While these initial studies showing the clear role for genetics in determining adverse responses to primaquine, isoniazid and succinylcholine were in progress, the general importance of the area was increasingly recognised. Motulsky [16] published a key review on the relationship between biochemical genetics and drug reactions, which highlighted the adverse reactions to primaquine and succinylcholine, in 1957. The term pharmacogenetics was first used in 1959 by Vogel [17] and was soon adopted by others working in the field.

PHARMACOGENETIC FACTORS AFFECTING DRUG DISPOSITION (PHARMACOKINETIC FACTORS)

The best studied polymorphisms are those in genes relevant to drug disposition, especially drug metabolism. This area is often referred to as pharmacokinetics. In this section, pharmacogenetic information relevant to the two phases of drug metabolism (phase I and phase II) and also drug transporters is considered.

Pharmacogenetics of drug oxidation

Overview

Pioneering studies on drug metabolism, especially those in the laboratories of the Millers [18], and of Brodie and Gillette [19], during the 1950s, showed that many drugs undergo oxidative metabolism in the presence of NADPH and molecular oxygen in liver microsomes. In 1962, Omura and Sato [20] described cytochrome P450 from a rat liver microsome preparation as a haemoprotein, showing a peak at 450 nm in the presence of carbon monoxide and dithionite. Shortly afterwards, Estabrook and colleagues showed that cytochrome P450 had steroid hydroxylase activity [21] and further studies confirmed its role in the metabolism of drugs such as codeine, aminopyrene and acetaminide [22]. At this time, it was still assumed that cytochrome P450 was a single enzyme but evidence for multiple forms emerged in the late 1960s [23,24] with purification of a range of rat and rabbit enzymes achieved during the 1970s [25,26].

In the mid 1970s, independent metabolism studies on two newly developed drugs, debrisoquine and sparteine, in the U.K. by Robert Smith and in Germany by Michel Eichelbaum [27,28], resulted in findings indicating that some individuals were unable to oxidize these drugs, although the majority of individuals showed normal metabolism. These studies estimated that 10% of Europeans showed absence of activity and the term ‘poor metabolizer’ was first used. At this time, the enzyme(s) responsible for this absence of activity were not known, but further studies confirmed that the deficiency in metabolism of both drugs co-segregated [29] and that the trait was inherited recessively [30]. It became clear that a number of different drugs, including tricyclic antidepressants, were also metabolized by this enzyme [31]. Studies on human liver microsomes confirmed that the enzyme responsible was a cytochrome P450 [32,33] and this enzyme was then purified to homogeneity [34]. The availability of antibodies against the purified protein facilitated the cloning of the relevant cDNA [35]. On the basis of emerging results on cytochrome P450 genes, it was agreed that the gene encoding the debrisoquine/sparteine hydroxylase should be termed CYP2D6. Studies on human genomic DNA led to the identification of several polymorphisms in CYP2D6 associated with the poor-metabolizer phenotype, including the most common splice site variant, a large deletion and a small deletion [36–40]. An additional contribution to the field was made in 1993 by Johansson and colleagues who described the phenomenon of ultrarapid metabolizers with one or more additional copies...
of CYP2D6 present [41]. These ultrarapid metabolizers had been previously identified on the basis of a poor response to tricyclic antidepressants. The elucidation of the mechanism underlying this rapid metabolism was one of the first accounts of copy number variation in the human genome.

In a similar approach to that used in the discovery of the CYP2D6 polymorphism, Kupfer and Preisig [42] found that some individuals showed absence of metabolism of the anticonvulsant S-mephenytoin. It was demonstrated that S-mephenytoin metabolism did not co-segregate with that of debrisoquine and sparteine with this polymorphism being due to a separate gene defect. Identification of the gene responsible for S-mephenytoin hydroxylase proved difficult initially, probably because the relevant enzyme was expressed at a low level in the liver. The gene, now termed CYP2C19, was cloned in 1994 and the two most common polymorphisms associated with absence of S-mephenytoin hydroxylase activity identified [43, 44].

A number of other cytochrome P450 genes are now known to be subject to functionally significant polymorphisms. In the case of one of these, CYP2C9, which metabolizes a range of drugs including warfarin, tolbutamide and non-steroidal anti-inflammatory drugs, some evidence for the existence of a polymorphism appeared in 1979 when a trimodal distribution for tolbutamide metabolism was reported [45]. Subsequently, it was shown that tolbutamide metabolism was distinct from debrisoquine metabolism [46]. The enzyme metabolizing tolbutamide was purified and cloned, and later named CYP2C9 [47, 48]. Analysis of CYP2C9 cDNA sequences provided evidence for the presence of coding region polymorphisms resulting in amino acid substitutions, with expression studies suggested these were functionally significant [47, 49, 50]. Genotyping of patients undergoing treatment with warfarin, another CYP2C9 substrate, confirmed the functional importance of the two most common coding region CYP2C9 polymorphisms [51–53].

Current knowledge on cytochrome P450 polymorphisms

Collectively, the cytochromes P450 are the most important group of phase I metabolizing enzymes and it has been estimated that up to 80% of all prescribed drugs undergo oxidation reactions catalysed by these enzymes [54]. In terms of these drug metabolism reactions, CYP3A4 has the most important role and has been estimated to be responsible for approx. 50% of all cytochrome P450-mediated metabolism of prescribed drugs [55], with CYP2D6 and CYP2C9 responsible for approx. 25 and 20% respectively [56, 57]. More detail on drug substrates metabolized by these isoforms is provided in Table 1. There is also a smaller contribution to drug metabolism from CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C19 and CYP3A5. As summarized in Table 1, four CYPs that contribute to drug metabolism, CYP2D6, CYP2C19, CYP2A6 and CYP3A5, are subject to polymorphisms leading to absence of enzyme activity and in addition CYP2C9 activity is very low, although not completely absent, in some individuals due to two common polymorphisms. There are also a large number of polymorphisms leading to smaller changes in cytochrome P450 activities [55].

Current knowledge of phenotype–genotype relationships within the cytochrome P450 family is now more comprehensive than for the majority of human genes, although better understanding of some aspects, such as regulation of gene expression, is still needed.

As CYP3A4, CYP2D6 and CYP2C9 are the most important enzymes in terms of drug metabolism, pharmacogenetic factors affecting levels of these enzymes are considered in the present review in detail, together with those affecting CYP2C19, which is less important in terms of drug metabolism generally, but which has been well studied from the pharmacogenetic standpoint. CYP3A4 does not appear to be subject to polymorphisms that result in absence of activity, although it is well established that there is considerable inter-individual variation in overall levels of activity. This variability may be due to a number of different factors, including genetic polymorphisms. A substantial number of variant CYP3A4 alleles have now been described including some associated with non-synonymous mutations (see the CYP allele database; http://www.cypalleles.ki.se/). Several of these give rise to alterations in catalytic activity. However, the non-synonymous mutations that have been described are seen at low population frequencies and therefore seem unlikely to be able to explain inter-individual variation in CYP3A4 activity fully. A number of upstream polymorphisms have also been detected with one being common (−392A > G, the CYP3A4*1B allele). The precise functional significance of this polymorphism remains unclear, but one study has found significantly lower metabolism of quinine in carriers of this allele compared with wild-type individuals [59]. This may be due to decreased binding of nuclear proteins in the region of the polymorphism [59]. It is possible that inter-individual variation in hepatic levels of CYP3A4 could be explained in part by polymorphisms in one of its transcriptional regulators, the PXR (pregnane X receptor) or NR112, a member of the nuclear receptor superfamily (for a review, see [60]).

Some of the observed inter-individual variability in CYP3A4 levels could be due to inter-individual variation in ability to induce CYP3A4 as a result of polymorphisms in PXR and/or to inter-individual variation in levels of endogenous PXR ligands. Studies on polymorphism of PXR have identified several SNPs (single nucleotide polymorphisms) that appear to affect individual ability to induce CYP3A4 [61].

An additional genetic factor that may affect overall CYP3A-related metabolism is the CYP3A5 gene which is expressed in only approx. 10% of Europeans [62]. Polymorphisms in CYP3A5 that explain the basis of the variation in expression of this gene have been well studied. In particular, a polymorphic site in intron 3 results in a 6986A > G polymorphism. Individuals positive for G (the CYP3A5*3 allele) at this position do not express CYP3A5 due to the creation of a cryptic splice site that results in the incorporation of intron sequence in the mature mRNA, resulting in the production of a truncated protein [63]. This allele is very common in all ethnic groups examined up to date. CYP3A5*3, which also results in abnormal splicing, and CYP3A5*7, which has a frameshift, are rarer alleles, but appear to explain the absence of CYP3A5 expression in some African-Americans [63]. It remains unclear whether variable expression of CYP3A5 can explain the wide inter-individual variation seen in overall CYP3A activity towards a number of substrates.

Over 80 different allelic variants of CYP2D6 have been identified and characterized (CYP allele database). Approx. 95% of European poor metabolizers have two copies of any combination of four alleles termed CYP2D6*3, CYP2D6*4, CYP2D6*5 and CYP2D6*6, which each encode defective forms of CYP2D6 [64, 65]. The remaining 5% of poor metabolizers are homozygous or heterozygous for a range of different loss-of-function alleles, with each individual allele relatively rare. Most inactivating mutations in CYP2D6 are either point mutations resulting in splicing defects or deletions which lead to either a truncated protein or no protein at all being synthesized.

Many individuals fall into the category of intermediate metabolizers. This phenotype is particularly common in certain African regions and in East Asians. Intermediate metabolizers may be either heterozygous for one of the inactivating mutations described above or homozygous for alleles associated with...
impaired metabolism. The best studied alleles associated with impaired metabolism are CYP2D6*10, which is common in East Asia, and CYP2D6*17, which is common in African populations. Both alleles have non-synonymous polymorphisms that result in a less catalytically active gene product. A study of the activity of the variant enzymes with a range of substrates has found that the extent of difference in catalytic activity between these isoforms and the reference ‘wild-type’ variant depends on the individual substrate [66]. In European populations, two alleles associated with impaired metabolism, CYP2D6*9 and CYP2D6*41, are relatively common. CYP2D6*9 encodes a protein with an amino acid deleted. CYP2D6*41 includes several different polymorphisms, including two non-synonymous mutations which are also seen in the CYP2D6*2 allele, an upstream polymorphism at position −1584 and a base substitution in intron 6. The non-synonymous mutations characteristic of CYP2D6*2 appear not to affect enzyme activity, but the intron 6 polymorphism has been demonstrated to be associated with altered RNA splicing, leading to lower levels of protein [67].

Ultrarapid metabolizers were originally identified on the basis of their extremely fast clearance of the antidepressant desmethylimipramine. Some individuals in this category have 13 copies of CYP2D6, arranged as tandem repeats, but a single gene duplication event is more commonly associated with the ultrarapid phenotype [41]. Depending on the country of origin, 1–8% of Europeans have one extra copy of the CYP2D6*1 or CYP2D6*2 alleles resulting in faster than average metabolism [68,69]. Subjects with three to five tandem copies of CYP2D6*2 have also been detected, mainly in African populations [70].

It is now clear that the number of active CYP2D6 alleles present in an individual’s genome is highly predictive of actual CYP2D6 enzyme activity, with those heterozygous for poor metabolizer alleles showing lower levels of activity compared with those with two normal alleles. This gene-dose effect was illustrated by a pharmacokinetic study on the antidepressant nortriptyline (Figure 1), which confirmed the intermediate level of metabolism in heterozygotes and also the increased rate of metabolism typical of ultrarapid metabolizers [71].

Despite the fact that the CYP2D6 polymorphism was initially identified over 30 years ago and that it has been possible to identify most of those with the genetic deficiency for the last 20 years, CYP2D6 genotyping has so far failed to enter routine clinical practice. There are a number of possible reasons for this.

### Table 1 CYP genes and polymorphic drug metabolism

<table>
<thead>
<tr>
<th>Isoform</th>
<th>Examples of drug substrates</th>
<th>Nature of polymorphism</th>
<th>Effect on activity</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A2</td>
<td>Clozapine, olanzapine theophylline and caffeine</td>
<td>Polymorphisms in non-coding sequences may affect expression or induction.</td>
<td>No absence of activity reported. Some effects on expression or induction.</td>
<td>[55,190]</td>
</tr>
<tr>
<td>CYP2A6</td>
<td>Coumarin and nicotine</td>
<td>Non-synonymous mutations, large deletion and upstream polymorphisms.</td>
<td>Absence of activity seen at low frequency. Ultraparapid metabolizers may also occur.</td>
<td>[55,190]</td>
</tr>
<tr>
<td>CYP2B6</td>
<td>Cyclophosphamide and efavirenz</td>
<td>Non-synonymous and upstream polymorphisms.</td>
<td>No absence of activity reported. Variation in activity common.</td>
<td>[55,191,192]</td>
</tr>
<tr>
<td>CYP2C8</td>
<td>Paclitaxel, retinoic acid, rosiglitazone and repaglinide</td>
<td>Non-synonymous and upstream polymorphisms.</td>
<td>No absence of activity reported. Variation in activity common.</td>
<td>[55,190,193]</td>
</tr>
<tr>
<td>CYP2C9</td>
<td>Warfarin, ibuprofen, diclofenac, tolbutamide and phenyltin</td>
<td>Non-synonymous polymorphisms.</td>
<td>Very low activity in some individuals.</td>
<td>[55,57,190,194,195]</td>
</tr>
<tr>
<td>CYP2C19</td>
<td>Omeprazole, diazepam, clozapam and clonipidogrel</td>
<td>Also upstream polymorphisms.</td>
<td>No absence of activity reported. Some ultrarapid metabolizers.</td>
<td>[55,92,94]</td>
</tr>
<tr>
<td>CYP2D6</td>
<td>Codeine, amitriptyline, nortriptyline, tamoxifen, metoprolol, timolol, tropisetron, dextromethorphan, atomoxetine, venlafaxine, zolpidem, zuclopenthizol, perphenazine, rosipenedone and haloperidol.</td>
<td>Splice site, initiation codon, non-synonymous and upstream polymorphisms.</td>
<td>Absence of activity common. Some ultrarapid metabolizers.</td>
<td>[55,190,195,196]</td>
</tr>
<tr>
<td>CYP2E1</td>
<td>Ethanol and isoniazid</td>
<td>Non-synonymous polymorphisms rare.</td>
<td>No absence of activity reported. Some variation in activity.</td>
<td>[55,190]</td>
</tr>
<tr>
<td>CYP3A4</td>
<td>Midazolam, cyclosporine, tacrolimus, erythromycin, nifedipine, simvastatin atorvastatin, diltiazem verapamil, vircristine and dapsone</td>
<td>Upstream polymorphisms common.</td>
<td>No absence of activity reported. Some variation in activity.</td>
<td>[55,197,198]</td>
</tr>
<tr>
<td>CYP3A5</td>
<td>Broadly similar to CYP3A4 above</td>
<td>Splice site polymorphisms common.</td>
<td>Absence of activity common.</td>
<td>[55,62,197,198]</td>
</tr>
</tbody>
</table>

### Figure 1 Pharmacokinetics of nortriptyline in healthy volunteers of defined CYP2D6 genotype

The study involved administration of a single oral dose of nortriptyline (25 mg) followed by blood sampling at the time points shown. The number of active CYP2D6 alleles was determined by genotyping and is shown in the Figure. Adapted from Dalen et al. [71] with permission from Macmillan Publishers Ltd. Clinical Pharmacology and Therapeutics © 1998. (http://www.nature.com/clpt/).
These include the general difficulty of introducing genetic tests into clinical practice, the fact that a number of key CYP2D6 substrates have been withdrawn from the market due to the problems experienced by poor metabolizers (e.g. phenformin and perhexiline) and that certain classes of CYP2D6 substrates, such as the tricyclic antidepressants, are less commonly used than when the polymorphism was first described. Guidelines for dose adjustment for antidepressant drugs on the basis of the CYP2D6 genotype have been formulated, but have not yet been tested in clinical trials [72]. However, there is now increasing new evidence that the CYP2D6 genotype may be relevant to the outcome of treatment with codeine and tamoxifen.

The widely used analgesic drug codeine is an important CYP2D6 substrate. It is activated to morphine exclusively by CYP2D6 and this is generally accepted to be essential to achieve analgesia (for a review, see [73]). Two case-reports concerning excessive activation of codeine in ultrarapid metabolizers with one additional copy of CYP2D6 have appeared. In the first, a patient prescribed a cough medicine containing codeine suffered life-threatening opioid intoxication [74]. When genotyped, this patient was found to have at least three copies of CYP2D6. The second concerned the death of a breast-fed baby 13 days after birth [75]. His mother was prescribed codeine as an analgesic post-delivery. Post-mortem examination of stored breast milk samples showed a morphine level at least 4-fold higher than expected. The mother was found to have a CYP2D6 gene duplication, which would explain the higher than expected morphine level in her milk, but the infant was an extensive metabolizer. A study on codeine administration to healthy volunteers of known CYP2D6 genotype showed that ultrarapid metabolizers were significantly more likely than extensive metabolizers to suffer sedation [76]. It therefore appears that in patients requiring treatment with codeine and related compounds, CYP2D6 genotyping followed by dose adjustment or prescription of an alternative drug could be beneficial in both avoiding dangerous intoxication and lack of response. There are recent data from a case-control study suggesting that babies of mothers with ultrarapid metabolizer CYP2D6 genotypes, who use codeine as an analgesic, are more likely to suffer central nervous system depression than other babies [77]. However, there is also a possibility that high levels of morphine described in a case-report on neonatal death could have resulted from concurrent intake of codeine and heroin rather than a problem CYP2D6 genotype [78].

Tamoxifen is a widely used treatment for hormone-receptor-positive breast cancer. Its metabolism is complex but it has been recognized that CYP2D6 produces a 4-hydroxy-N-desmethyltamoxifen metabolite (endoxifen) [79]. Endoxifen is found at high plasma levels in many patients and appears to bind strongly to oestrogen receptors suggesting it is important in the biological response to tamoxifen [80]. There is now considerable evidence that patients positive for two CYP2D6 poor metabolizer alleles show an increased incidence of breast cancer relapse [81–83].

In the case of CYP2C9, at least 33 variant alleles have now been identified (CYP allele database). With the exception of two of these (CYP2C9*6 and CYP2C9*31), all the described CYP2C9 variant alleles giving rise to decreased activity are associated with a single non-synonymous base change. The two most common variant alleles are CYP2C9*2 and CYP2C9*3 and, among Northern Europeans, over 30% of the population are positive for one or two of these alleles. An overall allele frequency for CYP2C9*2 of 10% compared with 8% for CYP2C9*3 was found in Europeans [53,84]. Both CYP2C9*2 and CYP2C9*3 occur more rarely in other ethnic groups including East Asians and African-Americans and the other variant alleles are all seen at population frequencies in the order of 1% [85].

CYP2C9 polymorphisms are particularly relevant to the metabolism of drug substrates with narrow therapeutic indices, where ADRs are common, such as to warfarin and phenytoin. In the case of warfarin, individualization of dose on the basis of drug response is a standard procedure, but a large number of studies have now found a consistent relationship between warfarin dose requirement and the CYP2C9 genotype [86]. In general, studies suggest that the CYP2C9 genotype contributes between 10 and 20% of the variability in warfarin dose requirement compared with a 20–30% contribution from the VKORC1 (vitamin K epoxide reductase complex 1) genotype [87] (see the section on Drug target polymorphisms below).

As discussed above, the phenotypic polymorphism affecting the metabolism of the anticonvulsant drug mephenytoin was described in the early 1980s [42]. The enzyme responsible for S-mephenytoin hydroxylation was later shown to be encoded by the CYP2C19 gene and two relatively common variant alleles associated with absence of enzyme activity were identified [43,44]. Other rarer alleles associated with either no activity or decreased activity were identified subsequently (CYP allele database). The two common alleles are associated with production of truncated proteins, although absence of activity can also arise due to amino acid substitutions [88]. Individuals with the CYP2C19 deficiency have two variant alleles present, although heterozygotes may show impaired metabolism. CYP2C19 is responsible for the metabolism of a relatively small number of commonly prescribed drugs, including the proton pump inhibitor omeprazole. Individuals with an absence of CYP2C19 activity show a better response to treatment of peptic ulcer with this drug compared with those with one or two normal alleles [89], apparently due to higher drug levels in the poor metabolizer group. Several recent studies indicate that the antplatelet agent clopidogrel is less effective in individuals with at least one defective CYP2C19 allele probably because of a major role for CYP2C19 in activation of this produg [90–93]. Some benzodiazepines including diazepam and cllobazam are CYP2C19 substrates and individuals defective in CYP2C19 may be at risk of toxicity, such as over-sedation [94]. The antidepressants citalopram and escitalopram are mainly metabolized by CYP2C19 and patients heterozygous for variant alleles show higher serum levels of the parent drug [95]. The variant allele CYP2C19*17 includes an upstream polymorphism that apparently increases transcription levels and is associated with higher levels of gene expression [96]. This variant appears to be associated with faster than normal metabolism of omeprazole and the antidepressant escitalopram [97,98].

Despite a good understanding of the relationship between phenotype and genotype for cytochromes P450, and the likely relevance of some genetic polymorphisms to the outcome of drug treatment, genotyping in patients undergoing treatment with drugs known to be substrates for cytochromes P450 associated with altered metabolism is still very limited. However, there is increasing interest in CYP2C9 genotyping prior to treatment with warfarin [99] (also see the section on Polymorphisms in target enzymes below). Guidelines for dose adjustment on the basis of the CYP2D6 and CYP2C19 genotype for a number of drugs used in psychiatry have also appeared [72], and there is considerable evidence that CYP2D6 deficiency may affect the outcome of tamoxifen treatment [83] (see above). The recent observation of a poorer response to clopidogrel treatment in CYP2C19-deficient patients has suggested genotyping for polymorphisms in this gene prior to prescription of clopidogrel may be of value [92].
Table 2 Phase II polymorphisms relevant to drug response

<table>
<thead>
<tr>
<th>Gene</th>
<th>Substrates</th>
<th>Polymorphism</th>
<th>Effect</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>UGT1A1</td>
<td>Bilirubin and irinotecan</td>
<td>Upstream TA insertion or non-synonymous SNP in exon 1</td>
<td>Decreased activity</td>
<td>[199,200]</td>
</tr>
<tr>
<td>UGT2B7</td>
<td>Morphine</td>
<td>Upstream and non-synonymous SNPs</td>
<td>Possible decrease in activity</td>
<td>[200]</td>
</tr>
<tr>
<td>NAT2</td>
<td>Isoniazid</td>
<td>Non-synonymous SNPs</td>
<td>No activity</td>
<td>[201,202]</td>
</tr>
<tr>
<td>TPMT</td>
<td>Mercaptopurine</td>
<td>Non-synonymous SNPs</td>
<td>No activity</td>
<td>[203]</td>
</tr>
</tbody>
</table>

Non-cytochrome-P450-mediated drug oxidation

Functionally significant polymorphisms have been described in genes encoding other enzymes of phase I metabolism in addition to the cytochromes P450, including the flavin-linked mono-oxygenases, esterases, amine oxidases and dehydrogenases. However, in the majority of cases, these polymorphisms affect enzymes with only a minor involvement in metabolism of prescribed drugs and are not considered further in the present review.

Pharmacogenetics of drug conjugation

As discussed in the Introduction section, a polymorphism affecting conjugation of drugs, such as isoniazid with acetyl-CoA, had been known to exist since the 1950s. Other polymorphisms affecting conjugation reactions of phase II metabolism were subsequently described from phenotyping studies. In particular, Weinshilboum and Sladek [100] identified several polymorphisms affecting methylation of xenobiotics and endogenous compounds by measurement of enzyme activities in blood cells. Those authors described the most pharmacologically important of these methylation polymorphisms, in TPMT (thiopurine methyltransferase), in 1980; approx. one in every 300 Europeans lack this enzyme, with lower activity observed in patients being prescribed 6-mercaptopurine or azathioprine and the UGT1A1*28 allele in patients receiving irinotecan is now recommended, but not mandated, by the U.S. Food and Drug Administration; knowledge of genotype can enable either dose adjustment or an alternative drug to be prescribed.

Pharmacogenetic studies on drug transporters

In addition to phase I and phase II metabolizing enzymes, drug transporters also contribute to drug disposition and their pharmacogenetics has been well studied in recent years. Transporter proteins that have roles in both inward and outward transport of drugs and their metabolites in cells relevant to drug disposition, such as hepatocytes, are of considerable pharmacogenetic interest. Those concerned with outward drug transport are mainly members of the ATP-dependent ABC transporter (ATP-binding-cassette transporter) family. Reports of a number of polymorphisms affecting the function of the well-characterized ABCB1 gene encoding the transporter P-glycoprotein first appeared in 2000 [112]. There have been a large number of follow-up studies on the functional consequences of polymorphism in ABCB1, although the literature remains unclear on the precise consequences of these polymorphisms, especially the well studied 3435G>T, a synonymous polymorphism that has been suggested to affect mRNA stability [113]. The relevance of ABCB1 genotype to drug disposition remains somewhat unclear. Pharmacogenetic studies on other ABC transporters, such as ABCB2, ABCB4 and ABCB11, have also been reported; there is limited evidence that certain coding and non-coding ABCB2 polymorphisms are functionally significant [114] and clearer evidence that rare variants in ABCB4 and ABCB11 contribute to inherited forms of cholestasis [115].

The drug transporters of the SLCO/OATP (solute carrier organic anion transporter) family, which are mainly concerned with the inward transport of drugs into cells like hepatocytes and renal tubular cells, have also been the subject of recent pharmacogenetic analysis. The anionic transporters of the SLCO1 family have been extensively characterized and the genotype for certain variants appears to be clinically important [116]. SLCO1B1 codes for the transporter OATP1B1, which transports anionic drugs into cells and is found in the hepatocyte sinusoidal membrane. A non-synonymous polymorphism in SLCO1B1 appears to affect the pharmacokinetics of several widely used drugs [116]. These include some, but not all, statins and this polymorphism may also be relevant to statin-related ADRs substitutions, was achieved in 1996 [109,110]. The most common variant allele giving rise to Gilbert’s syndrome was found to be a TA insertion in the promoter region of the UGT1A1 gene (UGT1A1*28 allele), which encodes the major UGT responsible for bilirubin conjugation [111].

Table 2 summarizes the main phase II polymorphisms that appear to be most relevant to drug metabolism on the basis of current evidence. Genotyping for the TPMT polymorphisms in patients being prescribed 6-mercaptopurine or azathioprine and the UGT1A1*28 allele in patients receiving irinotecan is now recommended, but not mandated, by the U.S. Food and Drug Administration; knowledge of genotype can enable either dose adjustment or an alternative drug to be prescribed.
(see the section on Idiosyncratic ADRs below). SLC22A1 and SLC22A2 (where SLC is solute carrier) encode the cationic transporters OCT1 (organic cation transporter 1) and OCT2. These transporters play important roles in transport of cationic drugs into hepatocytes and renal tubule cells respectively. There is increasing evidence that coding region polymorphisms in these genes are relevant to metformin response and to cisplatin-induced nephrotoxicity [117,118].

**PHARMACOGENETIC FACTORS AFFECTING DRUG TARGETS (PHARMACODYNAMIC FACTORS)**

Progress on the pharmacogenetics of drug receptors and other targets, such as enzymes and neurotransmitter transporters, has been slower than studies on drug metabolism and transport mainly because phenotypic evidence for the existence of functionally significant polymorphisms was generally not available. However, data from the human genome sequencing project has provided new insights into this area.

**Polymorphisms in receptors**

In the case of receptors, the majority of studies have focussed on metabotropic receptors, which are G-protein-coupled receptors with a characteristic seven-transmembrane domain structure, particularly dopamine, 5-HT (5-hydroxytryptamine, also known as serotonin) and adrenergic receptors. Pharmacogenetic information on other receptor types, such as the ionotropic nicotinic acetylcholine receptor or steroid hormone receptors, is still very limited and will not be considered further. Polymorphisms in the various adrenergic receptors have been demonstrated to be of considerable relevance to drug response, especially in the case of the β2-adrenergic receptor [119].

The relationship between polymorphisms in dopamine and 5-HT receptors and drug response is still less well established, although overall knowledge on variation in these receptors is extensive [120]. This section is therefore mainly concerned with polymorphisms affecting β-adrenergic receptors.

β-adrenergic receptors are the major adrenoreceptor class found in the heart with a key role in regulation of heart rate. The ADRB1 gene has a common non-synonymous polymorphism leading to a substitution at codon 389 (G389A) that has been well studied. Although the first cDNA clone isolated encoded the glycine variant, the frequency of the arginine variant is higher (approx. 70% in Europeans) [121,122]. For the Arg389 variant, transfection studies demonstrated slightly higher basal levels of expression compared with the glycine form, but when stimulated with isoproterenol, the arginine variant showed approx. 3-fold higher levels of activity [121].

As the G389A polymorphism is common and clearly functionally significant, it has formed the basis for a large number of clinical studies, particularly on the relationship between genotype and response to β-receptor antagonist treatment. Several studies have shown significant differences in response on the basis of genotype, both in studies on hypertension and on heart failure. A general conclusion from these studies is that patients homozygous for the Gly389 variant may show a poor response to β-antagonists and might benefit from higher drug doses, but, as suggested recently [123], larger prospective studies are needed to assess the clinical relevance of this concept.

The β2-adrenergic receptor is particularly well studied from the pharmacogenetic standpoint, probably due to its importance as a target for β-agonists in asthma. The receptor is encoded by the ADRB2 gene, which appears unusually polymorphic compared with other adrenergic receptors, especially in the coding sequence; this has resulted in considerable interest in assessing the relevance of these polymorphisms to drug response.

There are at least nine polymorphisms in the coding region with four of these non-synonymous polymorphisms. In addition, there is a single non-synonymous polymorphism in the region encoding the leader peptide and a number of upstream polymorphisms close to the transcription start site. A limited number of haplotypes have been observed with only four showing frequencies above 5% in any population studied to date, but there is considerable variation between haplotype frequency in different ethnic groups, especially those of European, African and East Asian origin [124]. Among Europeans, three haplotypes are common. Two of these differ mainly at codon 16 with one sequence encoding a glycine residue and the second an arginine residue at this position. Most clinical studies have focussed on the codon 16 polymorphism, probably because this SNP is seen at a relatively high population frequency and also was the first coding region polymorphism to be identified.

Although the codon 16 polymorphism was identified over 15 years ago, data on its functional significance is quite limited and rather contradictory. Early studies involving transfection of cell cultures suggested that the Gly389 variant showed enhanced down-regulation following agonist binding compared with the Arg389 form [125]. This down-regulation is the main mechanism by which long-term agonist-promoted desensitization of β2-adrenergic receptors occurs [126]. More recently, in a number of separate studies using either cells (lymphocytes and lung cells) of known genotype or genotyped patients receiving β2-adrenergic receptor-agonist treatment for asthma, it was found that the Arg389-containing form of ADRB2 was associated with increased agonist-induced down-regulation and with a poorer response to inhaled short-acting beta agonists [127–130]. The poorer response to β2-adrenergic receptor agonists appears to be specific to short-acting agonists as a large study involving patients being treated with longer-acting agonists and with an inhaled steroid for asthma failed to show any difference in outcome on the basis of the codon 16 genotype [131]. In general, it is now accepted that the codon 16 genotype does appear to affect response to short-acting agonists, but that this may not be clinically important as current treatments mainly involve use of longer-acting agonists. Further studies would be useful in resolving the question of whether genotyping patients taking β2-adrenergic receptor agonists for polymorphisms in ADRB2 and personalizing their treatment on this basis is of benefit.

The ADRB2 genotype may also be relevant in the response to β-adrenergic receptor antagonists. However, as most antagonists tend to have equal affinities for β1 and β2-receptors, or preferential interaction with β1 receptors, assessing the contribution of ADRB2 genotype to the response is more difficult. However, a few studies have studied this aspect in patients receiving β-adrenergic receptor antagonists for treatment of heart failure or hypertension. Most studies, especially larger more recent ones, have failed to detect any difference in response in relation to genotype for either codon 16 or codon 27 of ADRB2 [132–134].

**Polymorphisms in target enzymes**

As well as contributing to drug metabolism, enzymes are important drug targets. Widely used drugs targeting enzymes include the coumarin anticoagulants, which inhibit vitamin K epoxide reductase, ACE (angiotensin-converting enzyme) inhibitors, statins which inhibit HMG-CoA (3-hydroxy-3-methylglutaryl-CoA) reductase and nonsteroidal anti-inflammatory drugs which inhibit prostaglandin H-synthases I and II.
Vitamin K epoxide reductase, the target for coumarin anticoagulants, including warfarin, is another example of a gene with well-established pharmacogenetics. Limited phenotypic data from the 1970s suggested that the warfarin response was subject to inter-individual variation with resistance to the drug occurring in some families [135]. The gene encoding this enzyme VKORC1, was identified only in 2004 [136,137], but this was followed quickly by identification of isolated mutations associated with warfarin resistance and also common genetic polymorphisms affecting the response to anticoagulants [138–140]. Dosing with warfarin and other coumarin anticoagulants involves titration of dose until the desired level of anticoagulation is achieved. The dose required to achieve stable anticoagulation is subject to considerable inter-individual variation with a more than 100-fold variation seen. It is now clear that the genotype of VKORC1, and also of the cytochrome P450 CYP2C9 (see the section on Pharmacogenetics of drug oxidation above) accounts for up to 40% of the inter-individual variation in coumarin anticoagulant dose with age, body surface-area and interfering concomitant medications together accounting for an additional 15–20% of variation (for reviews, see [86,141]). Figure 2 demonstrates that VKORC1 and CYP2C9 genotypes are each separate genetic predictors of warfarin dose requirement. There are recent recommendations in the U.S.A. that genotyping for CYP2C9 and VKORC1 may be helpful in setting warfarin dosing and clinical trials comparing dosing tailored to genotype are underway in several countries [99].

HMG-CoA reductase converts HMG-CoA into mevalonic acid, which is the rate-limiting step in the biosynthesis of cholesterol [142]. It is also the target enzyme for the statins, which act to lower plasma cholesterol levels. In view of the large number of patients worldwide now taking statins, the possibility that pharmacogenetic factors, especially those affecting the target, might determine response is of considerable interest. Two polymorphisms in non-coding regions of HMGCR, the gene encoding HMG-CoA reductase, are associated with a decreased response to statin treatment and form part of a relatively rare haplotype including a polymorphism in the 3′-untranslated sequence which may affect RNA stability [143]. The haplotype frequency is approx. 7% in the US population, but is more common in African-Americans than in white Americans [144]. The low haplotype frequency means that, to date, it has only been possible to study statin response in heterozygotes, due to homozygous mutants being extremely rare. The original study reported that this haplotype results in a significantly smaller reduction in both total cholesterol and LDL (low-density lipoprotein)-cholesterol in response to pravastatin treatment and several more recent studies have attempted to replicate this for both pravastatin and other statins [143–148]. Only two of the five more recent studies have confirmed the original findings [144,148], but there are limitations with several of the other studies.

Pharmacogenetic studies on drug response have also been performed on other genes coding for drug targets, such as ACE [149] and the prostaglandin H synthases [150]. However, these are somewhat contradictory.

PHARMACOGENETICS OF ADRs

Idiosyncratic reactions, often referred to as type B ADRs, which are not directly predictable from drug concentration [151], are difficult to study because of their rarity, but their potentially very serious consequences for the patient makes them an important current research area in pharmacogenetics. The immune system, especially the products of class I and II HLA (human leucocyte antigen) genes, often contributes to idiosyncratic reactions, but this is not the case for all reactions of this type [152]. ADRs linked to drug concentration (type A reactions) are more common than idiosyncratic reactions and are also important clinically. These are more likely to be associated with polymorphisms affecting drug metabolism or drug transport.

As summarized in Table 3, there are a number of different types of idiosyncratic ADRs. Usually particular drugs are associated with one class of toxicity, although there are some examples where more than one type of toxicity can occur with a particular drug. Examples of commonly prescribed drugs linked to the different toxicities are also provided.
DILI (drug-induced liver injury) is a rare but clinically important problem. A U.S.-based study suggested that DILI accounted for 20% of all hospital admissions due to severe liver injury and 50% of acute liver failure cases, 75% of whom required a liver transplant [153]. Although the extent of the immune component in DILI is still not completely clear, a number of associations with particular HLA genotypes have now been reported. Initial reports of possible HLA associations with DILI appeared during the 1980s in relation to injury associated with nitrofurantoin, halothane and clometacin. More recently, two separate studies found a significant relationship between HLA class II genotype (the DRB1*1501 allele) and the susceptibility to liver injury induced by the antimicrobial agent co-amoxiclav [154,155]. Another antimicrobial agent, flucloxacillin, may also give rise to liver injury in some patients. Recent candidate gene and genome-wide association studies on flucloxacillin-induced liver injury (Figure 3) has detected a strong HLA association, with possession of the HLA-B*5701 allele associated with an 80-fold increased risk of DILI development [156]. The same HLA-B*5701 association has also been reported for another ADR, abacavir-related hypersensitivity [157] (see below), but has not been previously associated with DILI.

Two other HLA associations with DILI have also been described. One of these relates to the drug ximelagatran, which was found to be linked to liver toxicity in some patients during its development. From both genome-wide association and candidate gene studies, liver toxicity with this drug appears to be associated with the HLA allele DRB1*0701 [158]. This allele is more common among Europeans than in East Asians and the liver toxicity was also more common in Europe than in Asia. On the other hand, hepatotoxicity with ticlopidine appears to be more common among Japanese patients than Europeans and it has been shown that this is due in part to an association with HLA-A*3303, an HLA allele predominantly seen in East Asian populations [159].

These findings of HLA associations that seem to be drug-specific point to an important role for T-cell responses, possibly owing to drug complexed with peptides, in the toxicity process. The fact that DILI typically develops several weeks after the start of drug treatment is consistent with this immune component but it is likely that other factors, such as polymorphisms affecting drug metabolism and drug transport, also contribute and that not all DILI relates to HLA genotype.

Hypersensitivity refers to an inappropriate immune reaction to an otherwise non-toxic agent. The manifestations of hypersensitivity reactions are broad. Certain forms of DILI, as discussed above, can be regarded as hypersensitivity reactions, for example, co-amoxiclav-induced liver injury. Skin reactions, which may also involve other organs such as liver, lungs or kidneys, are the most common type of drug-induced hypersensitivity reactions.

Abacavir, a HIV-1 reverse transcriptase inhibitor, causes hypersensitivity reactions in 5% of patients [160]. These reactions can be fatal, particularly on rechallenge. An association between abacavir hypersensitivity and a haplotype including HLA-B*5701, HLA-DR7, and HLA-DQ3 was initially demonstrated by Mallal et al. [157] and then replicated in two other cohorts [161–163]. These findings have been confirmed further in a large randomized controlled trial [164]. The prevention of abacavir hypersensitivity using HLA-B*5701 genetic testing, which is now recommended prior to initiation of treatment in a number of different countries, is a prime example of translational research in pharmacogenetics.

CBZ (carbamazepine), a widely used anticonvulsant, can cause rashes in up to 10% of patients, and, occasionally, this may be the precursor to the development of a hypersensitivity syndrome [165,166]. CBZ can induce very rare blistering skin reactions such as SJS (Stevens–Johnson syndrome) and toxic epidermal necrolysis [167]. A study in patients from Taiwan has shown a very strong association between the HLA-B*1502 allele and CBZ-induced SJS [168]. The association seems to reflect the underlying frequency of the HLA-B*1502 allele. In Thai patients, where the population frequency of the allele is similar to that seen in Taiwan, an association between CBZ-induced SJS and HLA-B*1502 has also been demonstrated [169]. However, in Europeans [170,171] and Japanese [172] the allele frequency is lower, and no association has yet been shown between SJS development and HLA-B*1502.

Not all idiosyncratic ADRs show genetic associations with HLA. Susceptibility to two other important adverse factors, QT interval prolongation (representing an increased time for complete cycle of ventricular depolarization and repolarization in the heart’s electrical cycle) and statin-induced myopathy does not appear to show any immunogenetic association. In the case of statin-induced myopathy, there is evidence from a genome-wide association study (Figure 3) and an independent follow-up study that the genotype for SLCO1B1 (see the section on Drug transporters above) is a predictor of susceptibility to myopathy associated with at least with some statins [173,174]. This muscle toxicity, although very rare, is potentially fatal. SLCO1B1 genotype is also associated with plasma levels of several statins [175] and it therefore seems likely that the SLCO1B1 effect is at the pharmacokinetic level although additional genetic factors, unrelated to pharmacokinetics, may well also be predictors of toxicity.

Cardiotoxicity is another form of idiosyncratic ADR. Some drugs can rarely cause a significant lengthening in the QT interval, which is potentially fatal. Current knowledge on the genetic basis for susceptibility to this rare toxicity is limited, but a range of rare polymorphisms and mutations in ion channels appear to be associated with increased risk [176]. For example, a polymorphism in KCNE1, which encodes a voltage-gated potassium channel, is normally seen at a population frequency of 1% but is more common in individuals who have suffered drug-induced QT prolongation [177].

### Table 3 Common idiosyncratic adverse drug reactions

<table>
<thead>
<tr>
<th>Type</th>
<th>Drug examples</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>DILI</td>
<td>Co-amoxiclav, flucloxacillin and isoniazid</td>
<td>[204]</td>
</tr>
<tr>
<td>Drug-induced QT prolongation</td>
<td>Thioridazine, clarithromycin and terfenadine</td>
<td>[176]</td>
</tr>
<tr>
<td>Drug-induced hypersensitivity</td>
<td>Abacavir</td>
<td>[205]</td>
</tr>
<tr>
<td>Drug-induced muscle toxicity</td>
<td>Statins, chloroquine and penicillamine</td>
<td>[206]</td>
</tr>
<tr>
<td>Drug-induced serious skin rash</td>
<td>Carbamazepine and co-trimoxazole</td>
<td>[207]</td>
</tr>
</tbody>
</table>

**Genome-wide association studies in pharmacogenetics**

Increasingly, genome-wide association studies to identify genotypes associated with either drug response or drug toxicity are being performed. These studies involve genotyping disease cases and unaffected controls for typically between 500,000 and 1,000,000 SNPs, which represent variability in the entire human genome. Due to the existence of extensive linkage disequilibrium within the human genome, genotyping for particular polymorphisms (tag SNPs) will provide information on additional polymorphisms in linkage disequilibrium. By comparing distribution of polymorphisms in cases and controls, it is possible to identify particular polymorphisms where there is a difference in frequency. A difference in frequency suggests...
that variation in the gene where the polymorphism is located or one situated nearby may contribute to disease susceptibility. Owing to the large number of different polymorphisms studied, it is necessary to set a high threshold for statistical significance to avoid false positive results. In addition, any apparently positive associations are normally replicated in a second independent set of cases and controls. Most genome-wide associations on common diseases have involved in excess of 1000 cases and 1000 controls to provide sufficient statistical power to detect the small genetic effects typically seen. However, it is increasingly clear that some pharmacogenetic effects are larger and can be detected using smaller numbers of samples [178]. As reviewed elsewhere [179], genome-wide association studies have a completely open approach, allowing the detection of associations not directly predictable from existing knowledge and for the effects of a number of different genes to be detected simultaneously.

Genome-wide association studies have been widely reported for complex polygenic diseases, with some interesting novel genes affecting disease susceptibility now identified [180]. In the pharmacogenetics field, a number of genome-wide association studies on drug response or susceptibility to ADRs have appeared in the last 3 years. In some cases, these have confirmed existing findings from candidate gene studies performed previously and have not provided any additional novel findings, particularly in the case of two studies on warfarin dose requirement [181,182] and also, recently, in a study on clopidogrel response [91]. More generally, pharmacogenetic studies involving genome-wide association analysis have mostly pointed to only one or two genes having a major effect (see Figure 3 for two examples), rather than the larger number of genes, each with a small effect, as typically seen in the complex polygenic disease studies [179]. However, the open nature of the genome-wide approach has enabled some novel pharmacogenetic associations that would be unlikely to be detected by candidate gene studies to be identified. For example, the main current treatment for hepatitis C virus infection involves administration of a modified form of interferon-α and the antiviral drug ribavirin. Up to 50% of patients show a positive response to this treatment and become essentially virus-free. In three independent genome-wide association studies, polymorphisms adjacent to the IL-28B (interferon λ3) gene were shown to significantly predict the likelihood of a positive response to interferon-α [183–185]. In a study on bisphosphonate-induced osteonecrosis of the jaw, a CYP2C8 polymorphism gave the strongest effect in a genome-wide association analysis, even though bisphosphonates are not subject to metabolism [186]. This association may relate to a physiological role for CYP2C8 in inflammation [187], but this gene was not an obvious candidate for a role in this disease. As discussed in the section on idiosyncratic ADRs, a strong association between HLA-B*5701 and susceptibility to flucloxacillin-induced liver injury was detected by both direct HLA genotyping (performed because HLA genes were obvious candidates) and by a genome-wide association study (Figure 3) [156]. However, the genome-wide analysis also provided evidence for a role for the gene ST6GAL1, which encodes the enzyme α-2,6-sialyltransferase 1. This enzyme has a possible role in
REFERENCES

CONCLUSIONS

Over the 40 year period from 1957 to 1997, pharmacogenetics evolved to pharmacogenomics. There has been considerable further progress in the subsequent 14 years. Our understanding of single-gene effects, especially in relation to drug metabolism, is now comprehensive, but our understanding of effects from multiple genes is still more limited. In addition, we still need to translate the range of well-validated and clinically relevant pharmacogenetic discoveries that have been made over the years into more widespread use in patient care. Despite predictions that we are entering an era of personalized medicine [189], except for the few examples discussed above in relation to cancer and HIV treatment, this has not yet happened to any great extent. There is still considerable potential for using genomic approaches to individualize drug treatments, but the logistic and cost-effectiveness issues need to be addressed for these approaches to become routine.

B-cell immune responses [188], and would not be an obvious gene to study in relation to DILI although it is expressed in hepatocytes.

Pharmacogenetics and human genetic polymorphisms

445

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