Pharmacogenetics: an opportunity for a safer and more efficient pharmacotherapy

M. INGELMAN-SUNDBERG
From the Division of Molecular Toxicology, IMM, Karolinska Institutet, Stockholm, Sweden


Drug treatment is in many cases ineffective. Besides patients who do not respond to the treatment despite receiving expensive drugs, adverse drug reactions (ADRs) as a consequence of the treatment, is estimated to cost the US society 100 billion USD and over 100 000 deaths per year. Pharmacogenetics is the discipline which takes the patient’s genetic information of drug transporters, drug metabolizing enzymes and drug receptors into account to allow for an individualized drug therapy leading to optimal choice and dose of the drugs in question. It is believed that much cost for the society can be saved in this manner. Many drug transporters are polymorphic. In addition, the majority of phase I and phase II dependent drug metabolism is carried out by polymorphic enzymes which can cause abolished, quantitatively or qualitatively altered or enhanced drug metabolism. Stable duplication, multiduplication or amplification of active genes, most likely in response to dietary components that have resulted in a selection of alleles with multiple noninducible genes, has been described. Several examples exist where subjects carrying certain alleles suffer from a lack of drug efficacy because of ultrarapid metabolism caused by multiple genes or by induction of gene expression, or, alternatively, adverse effects from the drug treatment as a result of the presence of defective alleles. The information about the role of polymorphic drug receptors for efficiency of drug therapy is more scarce, although promising examples are seen in drug treatment of asthma where the efficiency can be severely enhanced by predictive genotyping of the drug targets. In addition, certain polymorphic genes can be used as markers for optimization of the drug therapy. It is likely that predictive genotyping is of benefit in 10–20% of drug treatment and thereby allows for prevention of causalities as a cause of ADRs and thus improves the health for a significant fraction of the patients. In 15–40% of the cases, the penetrance of genetic polymorphism is of less importance because of the polygenic influence on the outcome of drug treatment and in 50% of the cases, pharmacogenetics would be without influence because of other more important physiological and environmental factors. In the present contribution an overview about our present knowledge how polymorphic genes can influence the drug efficacy is presented. Some emphasis will be given to different forms of cytochrome P450 which are of importance for drug metabolism.

Keywords: adverse drug reactions, drug receptors, drug transporters, genetic polymorphism, poor metabolisers, ultrarapid metabolism.
Introduction

Interindividual variability in xenobiotic metabolism and drug response is extensive. The drug level in plasma can vary more than 1000-fold between two individuals having the same weight and with the same drug dosage. The causes for this variation are of genetic, physiological, pathophysiological and environmental origin. Genetic variability is known for drug absorption, drug metabolism and for drug interactions with the receptors. This forms the basis for slow and rapid drug absorption, poor, efficient or ultrarapid drug metabolism and poor or efficient receptor interactions (see Fig. 1). Environmental influence includes induction and inhibition of drug transport and metabolism. Inhibition caused by, for example, drug interactions is an important factor for the outcome of the drug plasma levels reached. Ageing is known to result in less capacity for drug metabolism as well as in less capacity to induce drug metabolizing enzymes. In the past decade, genetic factors for this variability have received much emphasis. One could envision that the genetic factors would account for about 20–40% of the interindividual differences in drug metabolism and response, but for certain drugs or classes of drugs, the genetic factors will be of utmost importance for the outcome of the drug therapy.

In the postgenomic area a lot of useful information is available that allows a genetic basis for better drug therapy and the discovery of new drug targets. The genetic information can be used for better pharmacotherapy and constitutes the research fields of pharmacogenetics and pharmacogenomics. Pharmacogenetics is best defined as the study of genetic variations that cause variable drug response and includes the genetic polymorphism of drug transporters, drug metabolizing enzymes and drug receptors. Pharmacogenomics is the research area which at the genome level aims at identifying disease genes

Fig. 1 Levels for interindividual variability in drug effects.

and new drug response markers at levels of drug absorption and metabolism, drug target or disease pathway.

There are about 30,000 different genes in the human genome. With a total of 3.12 billion nucleotides and the occurrence of single nucleotide polymorphisms (SNPs) at a frequency of 1/1250 bp between two individuals, one can estimate the total number of SNPs between two individuals to be about 2.5 million. Based on the genetic variability with respect to more rare SNPs taking hundreds of individuals into account, the actual number of SNPs might be between 10 and 15 million. Indeed more SNPs are facing the world each day because of the constant rate of new mutations introduced into the human genome.

The number of reported SNPs rapidly increases. In March 2000 only about 100,000 SNPs were present in the databases, but in April 2001 as many as 2,593,067 human SNPs were present (http://www.ncbi.nlm.nih.gov/SNP/) although only part of those are expected to represent true SNPs [1]. Of those, it is clear that the majority do not have any function and are mainly located between the genes, intergenic SNPs (iSNPs). Another class of SNPs are the perigenic SNPs (pSNPs) located in noncoding gene regions like the upstream regulatory regions, in introns as well as consisting of silent mutations, and between 200,000 and 500,000 pSNPs might be present in the genome. In the coding regions, the coding single nucleotide polymorphisms (cSNPs) cause alteration in amino acids and an estimated number of those are between 50,000 and 100,000. Thus, the entire phenotype would be dependent on the individual composition of those giving theoretically a possibility for, with the assumption of two base variations on each SNP, $2^{100\,000}$ which is equal to more than $10^{30\,000}$ number of different individuals. It is clear that knowledge of all these cSNPs would be of utmost importance for the understanding of the genetic basis for disease as well as for differential response to drug treatment.

In case of applications in pharmacogenetics, it is clear that the information we have today is substantial and allows providing the patients with information that could not only facilitate an individualized therapy both with respect to the choice of the drug, but also with respect to the dose of specific drugs. In my opinion, however, pharmacogenetics is still in the very beginning; knowledge about genetic variation at the level of drug metabolism is extensive, whereas the knowledge about interindividual differences in the function of drug transporters and drug targets is scarce. In addition, some of the results in this field are incomplete, conflicting and sometimes difficult to interpret. But the field as a whole is very promising and in the current review I consider some important achievements with emphasis on phase I enzymes. Some recent previous reviews in this field include those of Ingelman-Sundberg et al. [2], Meyer [3], Evans and Relling [4], and Spear et al. [5].

Adverse drug reactions and effectiveness of drug therapy

Adverse drug reactions is more of a problem in drug treatment and drug development than previously thought. A meta analysis revealed that serious ADRs occur amongst 6.7% of all hospitalized patients and that 0.32% of all hospitalized patients develop fatal adverse reactions, causing more than 100,000 deaths annually in the US [6]. Although this study was criticized for having many old studies amongst those causing the majority of deaths, subsequent follow up by the same authors, including studies in non-US countries, revealed a similar figure [7]. In a study in a major hospital Classen et al. identified 2227 instances of ADRs amongst hospitalized patients where the majority (42%) were because of miss-dosing and 50% had no preventable cause, likely to be related to genetic factors [8]. Of 1232 chemical entities approved as drugs in the USA 16% are associated with ADRs requiring a warning on the product label (Physicians Desk reference, 54th Edn, 2000). It has been estimated that ADRs causes up to 7% of all hospital admissions in the UK [9] and 13% of all admissions to internal medicine clinics in Sweden are caused by ADRs [10]. The costs for ADRs, including in average 2 days of prolonged hospitalization and reduced productivity, has been estimated to 100 billion US$ annually in the US [11].

In general, the efficiency of drug treatment is far from optimal (see Physicians desk reference, 54th Edn, 2000). The response rates in treatment of different diseases like Alzheimer’s, cardiac dysrhythmias, depression, incontinence, high blood pressure, osteoporosis, schizophrenia and rheumatoid arthritis
are in the range of 30–60%. In view of this relatively low frequency of responders and of the high cost and serious consequences of ADRs, it is attractive with the idea of personalized drug therapy. Identification of the true responders in each case would avoid treatment of the nonresponders resulting in less cost for the drug treatment per se, but also in reduced risk for ADRs with subsequent reduced costs for the society. As mentioned, the genetics of drug transporters, drug metabolizing enzymes and drug targets is critical to the understanding of a better drug therapy.

Drug transporters

Transporters of drugs that are expected to influence drug therapy are present in the intestine, in the blood brain barrier, intracellularly in, for example, CNS and in the liver and, in addition, in the kidney membranes. With respect to polymorphism of drug transporters, the clinical penetrance on drug treatment is less known. In total about 15 allelic variants of the MDR1 P-glyco-protein (P-gp) gene have been detected [12]. A synonymous mutation in MDR1 has been shown to associate to the rate of drug transport in the intestine [13], but the functional basis for this association was obscure as no functional mutation in the MDR1 gene was described. Recently, Kim et al. [12] found that a functional mutation Ala893Ser was often associated to the silent C3435T mutation (MDR1*2) detected by Hoffmeyer et al. [13] which might provide a functional explanation for the polymorphism seen. Indeed they have shown the existence of seven different MDR1 haplotypes, some of which causes altered function in a manner that was not compatible with the results of Hoffmeyer et al. [13]. Similarly Cascorbi et al. [14] found five missense mutations in the MDR1 gene, some of them very frequent in the Caucasian population, including the polymorphism at Ala893. It appears that many haplotypes of the P-gp are distributed within the populations and as yet no strict understanding regarding the importance of these variant forms for drug transport reactions in vivo are evident.

Recently seven different genes encoding drug transporters have been found in the MRP locus [15] but the genetic polymorphism of this locus has to be worked out. Recent screening for polymorphisms in other transporters has revealed missense mutations SLC6A4, encoding the serotonin transporter; and SLC18A2, encoding the vesicular monoamine transporter [16]. These results indeed indicate that the field of polymorphic drug transporters will explode in the near future. In general however, the effect of the mutations on the gross pharmacokinetics of a given drug would in many cases be difficult to interpret and be less in magnitude as compared with polymorphic drug metabolizing enzymes.

Drug metabolizing enzymes

Cytochromes P450

Sequencing of the human genome revealed 58 different human cytochrome P450 (CYP) genes according to David R Nelsons estimation (http://drnelson.utmem.edu/CytochromeP450.html). The majority of CYP genes encoding enzymes active in the metabolism of drugs and other xenobiotics are polymorphic and, in addition, a large number of pseudogenes are present. In fact it appears that only two of them, CYP1A1 and CYP2E1, are relatively well preserved and in essence no functionally important mutations are present in these genes.

The reason for this conservation might be the endogenous importance of the corresponding enzymes. CYP2E1 is a gluconeogenic enzyme and converts acetone to acetol which is subsequently converted to gluconeogenic precursors [17]. It is most plausible that a selection pressure has occurred because of its important role during conditions of extensive starvation. Furthermore the enzyme appears to play an essential role in fatty acid metabolism [18]. In case of CYP1A1, the physiological role is unknown. The Ah-receptor has, however, an important role in the cell cycle and it could be hypothesized that CYP1A1 might be an important mediator in some cell types. Concerning CYP2J2, CYP2R1, CYP2S1, CYP2U1 and CYP2W1, no polymorphism has yet been described but is likely to appear in the literature in the near future. Thus, the interindividual distribution of these P450 forms is strikingly different and the extensive polymorphism is most likely the result of dietary adaptation of different populations in the world. No important endogenous substrates have been described for any of
these polymorphic P450s and their primary function is most likely metabolism of dietary components.

In order to facilitate for scientists in the field, a web page has been created which contains continuously updated information regarding the polymorphic forms of CYPs (http://www.imm.ki.se/CYP-alleles). The aim of this page is to provide the scientists with a useful nomenclature for all enzyme variants with links to relevant literature describing the properties of the polymorphic enzymes. An important factor is also to bring the scientists up to date with the most recent knowledge so they can check whether allelic variants they have found have been described before. Presently a current update of the genetic polymorphism of CYP1A1, CYP1A2, CYP1B1, CYP2A6, CYP2B6, CYP2C6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4, CYP3A5, CYP3A7, CYP5A1 and CYP8A1 is presented. The field of polymorphic CYPs is quite complicated at present and, for example, more than 80 different alleles forms of CYP2D6 have now been described. A list of the most relevant variant forms of the CYPs of highest importance for the metabolism of drugs and other xenobiotics as well as their allele frequencies in Caucasians and Oriental populations are given in Table 1. As can be noted, most allelic forms are distributed with pronounced interethnic differences. The background for this is both a genetic drift and in some cases a genetic selection. Thus as a result of dietary stress in certain regions, specific CYP variants have been accumulated in certain populations (see below), whereas the majority of the interethnic differences are caused by incidental mutations in the genes, amplified in certain areas because of population expansion. The lack of endogenous function of the gene products has thus allowed this extensive heterogeneity with respect to allelic distributions in the different parts of the world.

The clinically most important polymorphism amongst hepatic P450s is seen in the CYP2C9, CYP2D6 and CYP3A4 genes, whose gene products account for 60–70% of all phase I dependent metabolism of clinically important drugs. With respect to CYP3A4, the variant alleles causing missense mutations are rare. However, at present the number of functionally different variants is increasing and amount to 20. A more frequent polymorphism in the CYP3A locus is observed for CYP3A5, having a similar substrate specificity as CYP3A4, and about 20% of the Caucasian population express high amounts of this enzyme. The basis behind this polymorphism was recently characterized and has been found to be the result of variant CYP3A5 genes causing several different splice variants, of course yielding inactive gene products [19].

Consequences of mutations in the CYP-genes

The mutations in the CYP genes can cause enzyme products with abolished, reduced, altered or increased enzyme activity. Abolished enzyme activity is commonly seen where the whole gene has been deleted, but has also its origin in mutations causing altered splicing, stop codons, abolished transcriptional start sites and deleterious amino acid changes. Mutations in substrate recognition sites (SRS) can cause the synthesis of enzymes with an altered substrate specificity as seen with CYP2D6*17 found entirely in black African populations and with CYP2C9*3. Furthermore, mutations in the folding region might lead to an altered protein folding and different substrate specificity as seen with CYP2D6*10 [20].

In vivo importance

As mentioned, pharmacogenetic and toxicogenetic studies rely very much on validation of the importance of the variant alleles in functional assays carried out both in vivo and in vitro. In my opinion mutations to be studied in the first place must create alterations in the gene products that are likely to affect function. Silent mutations or mutations in noncoding regions of dubious effects on expression should not primarily be investigated. Although such mutations might be linked to other of higher functional significance, there are always exceptions to such linkages and the study is then conducted using the wrong target, sometimes a waste of money and work.

A probe drug which is specific for the enzyme in question, that works in vivo, is most suitable for validation of results obtained in vitro. Drug monitoring using perphenazine, a CYP2D6 substrate, revealed that following a single dose of 6 mg, efficient metabolizers (EMs) obtained a serum level of 0.12 nM after 12 h, whereas poor metabolizers (PMs) lacking the CYP2D6 enzyme achieved 10 times the serum concentration and exhibited a very low clearance for perphenazine [21]. A similar
decrease in drug clearance for CYP2D6 PMs have been found for about 20 other drugs, being specific CYP2D6 substrates [2]. The PM phenotype of CYP2C19 causes a much larger effect of omeprazole treatment at the same dose as compared with that obtained in EMs. Thus, the increase in pH in the stomach at standard dosage is from 1.2 to 3 in EMs after 8 days of treatment with 20 mg of omeprazole daily, whereas in heterozygotes for a functional CYP2C19 enzyme the corresponding rise in pH was to 5.5 and in those homozygous for defective CYP2C19 pH raised to 6.2 with the same dose [22].

Table 1  Major human polymorphic cytochrome P450 enzymes

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Major variant alleles</th>
<th>Mutation</th>
<th>Consequence</th>
<th>Allele frequency (%)</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Caucas</td>
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<tr>
<td>CYP1A2</td>
<td>CYP1A2*1F</td>
<td>−164C &gt; A</td>
<td>Higher inducibility</td>
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<td></td>
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<td>C406Y</td>
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</tr>
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<td>CYP1A2*6</td>
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<td>Inactive enzyme</td>
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<td>Gene deletion</td>
<td>No enzyme</td>
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</tr>
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<td>TATA-mut</td>
<td>Less enzyme</td>
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<td>CYP2C9</td>
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<td>R144C</td>
<td>Reduced affinity for P450 reductase</td>
<td>8–13</td>
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<td>CYP2C9*3</td>
<td>I359L</td>
<td>Altered substr spec</td>
<td>7–9</td>
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<tr>
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<td>Inactive enzyme</td>
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<td>Inactive enzyme</td>
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<tr>
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<td>CYP2D6*2xm</td>
<td>Gene dupl</td>
<td>Increased activity</td>
<td>1–5</td>
</tr>
<tr>
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<td>Defective splice</td>
<td>Inactive enzyme</td>
<td>12–21</td>
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<tr>
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<td>Gene deletion</td>
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<td>1707T &gt; del</td>
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<tr>
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<td>CYP2D6*10</td>
<td>P148S, S486T</td>
<td>Unstable enzyme</td>
<td>1–2</td>
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<tr>
<td></td>
<td>CYP2D6*17</td>
<td>T107L, R296C, S486T</td>
<td>Reduced affinity for substrates</td>
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</tr>
<tr>
<td>CYP2E1</td>
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<td>R76H</td>
<td>Less enzyme expressed</td>
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<td>V389I</td>
<td>No effects</td>
<td>&lt;1</td>
</tr>
<tr>
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<td>CYP2E1*4</td>
<td>V179I</td>
<td>No effects</td>
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<td>S222P</td>
<td>Higher Km for subst.</td>
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<td>P218R</td>
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<td>831 insA</td>
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<td>G56D</td>
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<td>V170I</td>
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<td>D174H</td>
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<tr>
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<td>CYP3A4*11</td>
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<td>L373F</td>
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<td>P416L</td>
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<td>L135P</td>
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<td>R162Q</td>
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<td>No enzyme</td>
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<tr>
<td></td>
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<td>Splicing defect</td>
<td>No enzyme</td>
<td>Rare</td>
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</table>

*See2 and http://imm.ki.se/CYPalleles for details and literature references.
chromogranin A and lower pepsinogen I levels during long-term treatment of omeprazole [22]. This indicates that changes in gastric mucosal morphology during omeprazole treatment might be dependent upon the degree of the individual’s capacity to metabolize omeprazole.

Further important clinical effects in relation to the CYP polymorphism are the CYP2C9 polymorphism in relation to treatment with warfarin, CYP2D6 in relation to treatment with psychoactive drugs, in particular with tricyclic antidepressants which are almost entirely CYP2D6 substrates, and treatment with codeine, losartan and proguanil, where the active metabolite morphine is not formed amongst PMs. For examples, Daly and collaborators reported about increased bleedings amongst subjects carrying variant CYP2C9 alleles [23]. Dose requirements were here 0.5 mg day\(^{-1}\) amongst subjects with defective CYP2C9 alleles as compared with 5–8 mg amongst subjects with the wt alleles. A less extent of dose dependency on the CYP2C9 genotype but no increased frequency of bleedings amongst subjects with mutated CYP2C9 alleles were found by Taube et al. [24].

**CYP gene duplications**

In contrast to PMs, ultrarapid metabolizers (UMs) carry two or more active genes on the same allele. The gene effect is striking and clearance of nortriptiline or debrisoquine is proportional to the number of CYP2D6 gene copies [25, 26]. Gene duplications do not occur unless it is beneficial for the organism. This raises the question about the origin of CYP2D6 duplication, multiduplication and gene amplification, yielding alleles with 2, 3, 4, 5 or 13 gene copies. A summary of the interethnic differences reveals that a focus for CYP2D6 gene duplications has been in Ethiopia and Saudi Arabia with 20–30% of the population of this genotype (see Fig. 2). In

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Fig. 2 Interethnic distribution of alleles containing duplicated active CYP2D6 genes. Data are from Bernal et al. [66], Nishida et al. [67], Garcia-Barcelo et al. [68] as well as from Ingelman-Sundberg et al. [69, 70] and references therein.

other African countries like Ghana, Zimbabwe and Tanzania, this frequency is much lower, 2–8%, indicating a specific event causing this duplication in the area of Ethiopia and Saudi Arabia. Very few subjects with gene duplications are seen in Asia and Northern Europe. In the Mediterranean area about 10% of the population carry CYP2D6 gene duplications, most likely the result of a Muslim migration through Gibraltar about 700 AD. The distribution of the duplicated CYP2D6 genes world wide indicates that the gene duplication event has occurred rather recently, about 2000–5000 years ago.

The question arises whether stable gene duplication is a common phenomenon in humans. When examining the GSTM locus in the Saudi Arabian population, shown to have a very high frequency of CYP2D6 gene duplications [27], we found that subjects known to have a very high rate of conjugation with stilbene oxide, as measured in blood, carried a locus with two glutathione transferase M1 (GSTM1) genes [28]. The origin of this phenomenon is an unequal crossover event where one GSTM1 gene is transferred to the other allele leaving one as a null allele for GSTM1. Duplicated GSTM1 genes has yet not been described in any other population.

The basis for duplications is unequal crossover reactions. Thus it is anticipated that amongst loci known to be lacking specific genes encoding drug metabolizing enzymes the probability would be higher to find alleles with duplicated genes. A common variant of the CYP2A locus in Oriental populations lacks the CYP2A6 gene encoding for the major nicotine oxidase. This allele (CYP2A6*4) exists at a frequency of about 15% in Orientals, but is essentially lacking amongst Caucasians [29]. Sellers and collaborators [30] have provided evidence for the existence of alleles with duplicated CYP2A6 genes. The evidence is based on an unequal crossover point within the CYP2A6 gene and an increased amount of PCR products from subjects with duplicated CYP2A6 genes. It would, however, also be advantageous to have Southern blotting experiments as evidence for this locus. This locus (CYP2A6*1x2) was found in six subjects out of 455 examined, thus creating an allele frequency of 0.6%. Stable gene duplication thus seems to be a general phenomenon amongst the genes encoding drug metabolizing enzymes and more examples will be expected to come in the future.

A central question in this respect is the basis for conservation of alleles with duplicated genes. It is generally anticipated that gene duplication would not persist unless it is beneficial for the organism [2]. Cancer cells which are stressed with cytostatic agents can respond by gene amplification, but when the stress is removed the extra gene copies are being removed from the cells. As the origin of gene duplications mostly is the unequal crossover event, one can compare the frequency of null alleles and alleles with duplicated genes in order to understand more about the basis for the duplication. In the GSTM locus it is evident that the frequency of null GSTM1 alleles is 40–70% world wide whereas the alleles with duplicated genes are very rare. By contrast, about 4–5% of the CYP2D6 alleles are null alleles with a similar distribution world wide [2]. whereas in certain areas of the world the frequency of alleles with duplicated CYP2D6 genes reaches 15%. Thus, it is likely that preservation of duplicated CYP2D6 genes has been beneficial for survival in these regions, in particular in Ethiopia and Saudi Arabia. One might speculate about the origin of this event, but it appears likely that dietary stress might have played a central role. CYP2D6 is known to have a very high affinity for many alkaloids [31] and a higher potential for detoxification of plant toxins would be beneficial for survival under conditions of extreme starvation, which has frequently occurred in this area where CYP2D6 gene duplications are found at the highest frequency. The persistence of gene duplications after the stress has occurred is most probably related to the absence of any major endogenous function of the gene product, i.e. because an important fraction of the populations lacks the enzyme without showing any phenotype, a corresponding duplication of the gene would not be of influence on the endogenous function as to be able to have an impact on genetic normalization in the locus.

Clinical effects as related to gene duplications

Duplication of functional genes amongst the CYPs have been shown to cause the synthesis of more enzyme. Apparently, a feedback mechanism for the inhibition of gene transcription in subjects with multiple CYP genes has not been seen. Thus the rate of metabolism of either debrisoquine [32] or nor- triptyline [26] has been shown to be proportional to
the number of CYP2D6 gene copies. Similarly subjects carrying multiple CYP2A6 gene copies have been found to metabolize nicotine at a substantially higher rate [33]. Clinically this might be of a problem in certain cases. Thus, subjects with duplicated CYP2D6 genes taking codeine has suffered from severe abdominal pain and side-effects typical of the primary CYP2D6 dependent metabolite morphine [34]. In order to reach therapeutic levels of nortriptyline, the dosage has to be adjusted in relation to the number of gene copies. Thus amongst subject homozygous for a null CYP2D6 allele, the dosage should be 50 mg day\(^{-1}\), whether amongst subjects with multiple gene copies a dosage of 500 mg would be required in order to reach the therapeutic level [26].

**Other phase I enzymes**

Besides the P450 genes also other phase I enzymes are polymorphic. Functional significance has been unravelled for alcohol dehydrogenases, acetaldehyde dehydrogenases as well as for dihydropyrimidine dehydrogenase (DPD). With respect to the first two enzymes the clearance of ethanol is significantly affected. ADHB2 giving a higher rate for ethanol metabolism and ALDH2 polymorphism influencing acetaldehyde metabolism. PMs for ALDH2 develop flush reactions, and antabus like side-effects when drinking ethanol and the number of alcoholics in this genotype group is significantly lower than in subjects with the wt ALDH2 genotype.

A polymorphism relevant to treatment with anti-cancer drugs is present in DPD. The 5-fluorouracil is metabolized by this enzyme and subjects with impaired enzyme activity caused by inactivating gene mutations suffer from severely increased risk for ADRs including myelotoxicity and neurotoxicity [35].

**Phase II enzymes**

Extensive polymorphism does occur in the majority of phase II enzymes. However, only a few of these are of clinical or toxicological significance. To a great extent this is explained by a lower specificity for the substrates in question and an impressive arsenal of different forms of each enzyme class, but also by the fact that much fewer drugs are primarily metabolized by phase II enzymes. The most important polymorphisms from a functional and clinical point of view occur in glutathione transferase M1 (GSTM1), N-acetyltransferase-2 (NAT-2) and in thiopurinemethyl transferase (TPMT). Meta analyses regarding the relationship between the risk of receiving lung cancer and bladder cancer reveal a small increase in OR of about 1–2 for subjects without active GSTM1 [36]. Still, in my opinion, it is too early to conclude that such a relationship does exist. In case of NAT2 it appears that this polymorphism is related to ADRs upon treatment with isoniazide, dapsone and sulphoniazide. An increased risk for renotoxicity in response to isoniazide is seen in PMs for NAT2 as well as increased hypersensitivity to treatment with sulphonamines as well as hydralazine induced toxicity [37].

One of the more functionally important polymorphisms amongst the methyl transferases has been discovered and characterized by Richard Weinshilboum and William Evans [38]. People carrying defect TPMT alleles do metabolize the anticancer drugs 6-mercaptopurines at a much slower rate and, indeed, dosage optimization are dependent on the TPMT genotype. Amongst patients being homozygous for defect alleles 10 mg m\(^{-2}\) is an optimal dose as compared with 100 mg m\(^{-2}\) amongst subjects with wt alleles and also amongst heterozygotes dose reduction is required [39]. Data have accumulated indicating that the survival of patients with individualized dosage is better and, in fact, fatal outcomes of treatment of PM subjects with standard dosage of 6-mercaptopurines have been described [3].

**Polymorphic pharmacodynamics**

As mentioned, an increased knowledge is now provided regarding the functional consequences of polymorphic drug targets, although this area is by far not yet in a stage where clear cut recommendations for choices of drugs and drug dosages can generally be made based on the information about drug receptors (70% of the drug targets [40]), channels (30%) or specific enzymes (5% of drug targets).

\(\beta_2\)-adrenergic receptor

Amongst the most potentially interesting targets in development for genotyped-based pharmacotherapy is the \(\beta_2\)-adrenergic receptor. Functional mutations affecting the stability of the receptor have been found at Arg16. The Gly16 variant is down-regulated
to a great extent in response to ligand when expressed in recombinant cells. This has received support from in vivo studies where asthmatic children receiving albuterol had an increased bronchodilatation if they were of a Arg16 genotype consistent with the fact that this variant is less down regulated than the Gly16 variant. Other studies of different designs have however, failed to obtain such a relation. In addition, the polymorphism of Thr164 where the mutant Ile164 form has a decreased affinity for many, but not all, β₂- adrenergic receptor agonists is a clear example. A similar polymorphism is seen at Arg389 on the β₂-adrenergic receptor where the mutant form (Gly389) has lower coupling efficiency with isoproterenol [41].

A decrease in efficiency of a β-agonist during continuous use has revealed a decrease in peak expiratory flow rate in subjects homozygous for the Arg16 but not the Gly genotype which is in agreement that the Gly16 variant is not further down regulated by albuterol [41].

Studies of the β₂-adrenergic receptor is complicated by the fact that many upstream and coding region mutations occur, at least 13 different SNPs are present yielding a possibility of $2^{13} = 8192$ different haplotypes. However, in a study of 54 controls from three different ethnic groups and 121 Caucasian asthmatics, only 12 different haplotypes were shown to occur [42]. Some of the SNPs, in particular in the upstream region, were in close linkage, whereas those in the open reading frame were not. Examination of the haplotype in relationship to the effect of a β₂-agonist on the forced expiratory volume (FEV) revealed that subjects with some haplotypes in combination responded to a higher extent. In addition, there was a difference in expression of the receptor haplotypes in HEK293 cells both at the protein and mRNA level [42]. These results raise the possibility to use SNP haplotyping for predicting drug response as hypothesized by Roses [43], although the results with the β₂-receptor has to be reproduced by other investigators and more examples are needed to exemplify this principle before it is possible to consider this as a feasible manner for genotype-based drug prescription.

Serotonin 5-HT₂A receptor

Interesting associations between an His452Tyr polymorphism and clinical response of schizophrenic patients to the antipsychotic drug clozapine have been reported. In several studies, but not in all, the Tyr-allele was overrepresented amongst patients who were resistant to clozapine treatment [44–46]. Analysis for a combination of mutations in neurotransmitter related genes can give a high predictability for clozapine response [46]. See also Arranz et al. [47] for a recent overview about the situation of the role of pharmacogenetics for the individualization of psychiatric treatment.

The μ-opioid receptor

The μ-opioid receptor (MOR) mediates opiate induced euphoria, tolerance and dependence and regulate the effects of other substances with high addictive potential such as cocaine or ethanol [48]. MOR-deficient mice do not self-administer ethanol [49]. A functional polymorphism Ser268Pro in this receptor has been found which strongly impairs the receptor signalling and represents an interesting polymorphism in relation to drug addiction [50].

Long QT syndrome

Inherited long QT syndrome (LQTS) is a rare cardiac dysrhythmia that predisposes to torsades de pointes (TdP), ventricular defibrillation and sudden death [51]. Mutations in five ion channels causes the majority of cases of inherited LQTS. Acquired LQTS is a common disorder caused by drugs and metabolic abnormalities. These patients can have a genetic predisposition to dysrhythmia because of a mutation in the MinK-related peptide 1 (MiRP1) subunit of their I_{kr} potassium channels [52]. The channels with this subunit were three fold more sensitive to drug inhibition than wild type. Four different missense mutations have now been identified in MiRP1 and one (Ala116Val) is associated with antibiotic induced cardiac dysrhythmia [53].

Lipoxygenase A

A clear pharmacogenetic effect is seen amongst patients treated with leukotriene antagonists. Three major variant genes of Lipoxygenase A (ALOX 5) causing the presence of a variable number of binding sites for the transcriptional factor SP-1 have been found and treatment with zileuton and montelukast associates strongly with this polymorphism allowing
a clear identification of individuals being nonresponsive to treatment with these antagonists [54].

**Marker polymorphisms**

A polymorphic insertion of a 287-bp DNA fragment, possibly a silencer element, in the angiotensin-converting enzyme (ACE) gene accounts for a great extent of variability in the enzyme levels [55] and has been reported to associate to altered response to treatment with ACE-inhibitors in renal diseases and in hypertension and heart failure [56–59]. The functional link of this polymorphism still remains obscure and the results not entirely convincing about a predictive role of this polymorphism.

A marker genetic polymorphism in intron 1 (B1/B2) of the gene encoding cholesteryl ester transfer protein (CETP) has been studied in relationship to effect of the HMG-CoA reductase inhibitor pravastatin on decrease of the progress of the coronary atherosclerosis amongst 807 subjects and only individuals of the B1 genotype responded. High serum levels of CETP correlates to lower HDL serum concentrations and this might stimulate the development of arteriosclerosis. This polymorphism has also been linked to the progression of coronary artherosclerosis [60].

The allele for apolipoprotein E type 4 (ApOE-e4) is associated with the common late onset of familial as well as sporadic forms of Alzheimer’s disease [61]. The genetic locus for the disease has been located to chromosome 19, close to the APOE locus and the ApOE-e4 allele associates both with increased risk for Alzheimers and an earlier onset. Tacrine has been described to have less effect amongst subjects of the ApOE-e4 genotype [62].

**Pharmacogenetics – implications for the future**

The application of pharmacogenetics in research and drug development is not entirely straightforward. Many pharmacogenetic studies are not optimally designed. Association studies are often based on a patient material that is badly characterized, with too small number of individuals and patients not thoroughly phenotyped. Many studies concern synonymous mutations or marker SNPs with no established function. Sometimes inappropriate sampling strategies are used. A severe publication bias is often the result of these problems. Also pharmacogenetics meets concerns from patients who are worried about the information about their genome, from doctors who think that prescribing drugs become complicated and from industry who can think that the result of pharmacogenetics can be shrinking markets for each drug. The benefits for industry is however, that clinical trials in some cases can be smaller and just used in genetically defined subpopulations, and that screening for interactions with polymorphic enzymes early in the development process can reduce problematic dosing of future chemical entities because less drugs which are substrates for the polymorphic enzymes will be released to the market. It is evident that we have to consider these problems for the future and learn to make better studies which are based on a material with enough power and that fulfils the criteria for epidemiological and biological research of good quality. Another concern is the occurrence of SNPs in various combinations creating different haplotypes of which only a few indeed cause an altered function or expression of the gene product. Single SNP analysis can be of limited value, and still we do not have efficient methods for the determination of true haplotypes, particularly in large genes.

In general, one could divide the impact of pharmacogenetics into three classes. Class I constitutes the cases where a tight link between genotype and effect of drug treatment is achieved. Here certain mutations in a limited number of genes have a high penetrance for the outcome of the drug treatment. In my opinion, this would account for 10–15% of all cases for drug therapy today provided that we knew all important polymorphisms for the drug therapy in question. The second class represents cases where the drug therapy is dependent on polygenic factors. Because of polymorphism with opposite net effects the outcome for the drug therapy would be difficult to predict. In certain cases, however, the attention of several polymorphic loci might be beneficial for drug therapy in question. The second class represents cases where the drug therapy is dependent on polygenic factors. Because of polymorphism with opposite net effects the outcome for the drug therapy would be difficult to predict. In certain cases, however, the attention of several polymorphic loci might be beneficial for drug therapy. This class might represent 35–40% of all cases for drug treatment. In the third class (about 50% of the cases), the pharmacogenetic influence on drug therapy is insignificant, because of the action of gene products without any significant functional polymorphism. Here, drug interactions, physiological and pathological consequences are by far more important factors to consider as reasons for interindividual variability in the outcome of drug treatment.
Use of pharmacogenetics today

Pharmacogenetics is only in the very beginning with respect to use in clinical practice. Based on the very rapid increased knowledge about functionally important SNPs it is conceivable that we in the near future will have information that allows individualized drug therapy based on the genetic constitution at a much higher resolution, which can be effectively used for a safer and more efficient pharmacotherapy. In Table 2 some important examples are given where we can already apply pharmacogenetics. With respect to drug transport, the functional polymorphism of the MDR1 gene provides an important topic for further studies. In particular it is relevant to study the effect on drug distribution in the brain. Concerning phase I metabolism, genotyping provides an important tool for more efficient drug therapy using a limited number of specific drugs. These include warfarin (CYP2C9), tricyclic antidepressants (CYP2D6), codeine (CYP2D6), perphenazine (CYP2D6) for treatment of schizophrenia, and long-term treatment with omeprazole (CYP2C19) and related pump inhibitors. In addition, 5-fluorouracil treatment in relationship to the polymorphism of DPD is relevant for genotyping. Amongst the phase II enzymes, it is evident that predictive genotyping for deficiencies in the TPMT gene is very relevant for correct dosing of 6-mercaptopurines during treatment of leukaemia and that NAT2 genotyping is beneficial for prediction of side-effects caused by isoniazide and hydralysine. On the receptor level the most promising relationships have perhaps been found in asthma for treatment with β₂-adrenergic agonists with leukotriene antagonists and prediction of drug induced LQTS, clozapine response for treatment of schizophrenia as well as the use of ApOE-ε4 as a marker for tacrine treatment. In other cases with respect to drug metabolizing enzymes, more studies are needed before the impact

<table>
<thead>
<tr>
<th>Gene</th>
<th>Important polymorphism</th>
<th>Drugs</th>
<th>Consequence</th>
<th>Reference</th>
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</thead>
<tbody>
<tr>
<td>Transporters</td>
<td></td>
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<tr>
<td>MDR1</td>
<td>Ala893Ser and others</td>
<td>Many</td>
<td>Altered drug transport</td>
<td>[13, 14]</td>
</tr>
<tr>
<td>CYP2A6</td>
<td>Gene deletion</td>
<td>Nicotine</td>
<td>Addiction?</td>
<td>[29]</td>
</tr>
<tr>
<td>CYP2C9</td>
<td>Gene inactivation (PMs)</td>
<td>Losartan</td>
<td>Haemorrhage</td>
<td>[23]</td>
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<tr>
<td>CYP2C19</td>
<td>Gene inactivation (PMs)</td>
<td>Omeprazole</td>
<td>Less antihypertensive effect</td>
<td>[63]</td>
</tr>
<tr>
<td>CYP2D6</td>
<td>Gene inactivation (PMs)</td>
<td>Diazepam</td>
<td>Increased pH in the gut and increased therapeutic efficacy</td>
<td>[22, 64]</td>
</tr>
<tr>
<td>Phase II enzymes</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>N-acetyltransferase 2</td>
<td>Gene inactivation (PMs)</td>
<td>Sulphonamide</td>
<td>Hypersensitivity, toxicity</td>
<td>[37]</td>
</tr>
<tr>
<td>Thiopurine methyltransferase</td>
<td>Gene inactivation (PMs)</td>
<td>Hydralysine</td>
<td>Myelotoxicity</td>
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<td>Drug targets</td>
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<tr>
<td>β₁ adrenergic receptor</td>
<td>Arg1389Gly</td>
<td>Isoproterenol</td>
<td>Lower coupling efficiency</td>
<td>[41]</td>
</tr>
<tr>
<td>β₂ adrenergic receptor</td>
<td>Arg16Gly Thr164Ile</td>
<td>Receptor agonists</td>
<td>Decreased affinity</td>
<td>[41]</td>
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<tr>
<td>Serotonin 5-HT₂A receptor</td>
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<td>Clozapine</td>
<td>Lowered response</td>
<td>[4]</td>
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<td>MiRP1</td>
<td>Ala116Val</td>
<td>Antibiotics</td>
<td>Antibiotic induced arrhythmia</td>
<td>[53]</td>
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<td>Lipoxygenase A</td>
<td>SP-1-binding motif</td>
<td>Zileuton, montelokast</td>
<td>No drug effect</td>
<td>[54]</td>
</tr>
<tr>
<td>Marker</td>
<td>Mutation in the 5’-upstream region</td>
<td>Tacrine</td>
<td>Less response in Alzheimer’s patients</td>
<td>[62]</td>
</tr>
</tbody>
</table>
of genotyping for the efficiency of drug therapy can be validated. Correctly used pharmacogenetics can thus already be an invaluable instrument in many cases for better drug design, less adverse drug reactions, lower cost for drug treatment and for a better health. Because of the rapid development in the field it is believed that in already a couple of years from now, pharmacogenetics will be a standard tool in drug development and clinical practice.

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Correspondence: Dr Magnus Ingelman-Sundberg, Division of Molecular Toxicology, Karolinska Institutet, IMM, 171 77 Stockholm, Sweden (fax: +468-337-327; e-mail: maging@ki.se).