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Review Article

Pharmacogenetics: the science of predictive clinical pharmacology

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Abstract. The study of pharmacogenetics has expanded from what were initially casual family-based clinical drug response observations, to a fully-fledged science with direct therapeutic applications, all within a time-span of less than 60 years. A wide spectrum of polymorphisms, located within several genes, are now recognised to influence the pharmacokinetics and pharmacodynamics of the majority of drugs within our therapeutic armamentarium. This information forms the basis for the new development of pharmacogenetic genotyping tests, which can be used to predict the therapeutic and/or adverse effects of a specific drug in a particular patient. Pharmacogenetic-guided, patient targeted therapy has now become the developing fulcrum of personalized medicine, as it provides the best means to optimize benefit/risk ratio in pharmacological management.

Keywords Pharmacogenetics - targeted therapy - predictive biomarkers - personalized medicine.

1 Introduction

The understanding of molecular pathology of disease, together with the discovery of novel therapeutic targets, has greatly enhanced the evolution of new drugs. On the forefront of such discoveries lie the various biotech drugs produced by recombinant DNA technology, such as the anti-cancer drugs trastuzumab and ibrutinib; the latter being granted a license by the FDA in February 2014 (FDA, 2014); as well as recent receptor-specific ligands such as the angiotensin II receptor blocker azilsartan, which was approved by the FDA for use in hypertension management in February 2011 (FDA, 2011). Although scientific knowledge in this area has been increasing exponentially, it has frequently been observed that different patients often exhibit altered responses to such therapies. These responses may manifest as very high or very low drug efficacy, or as an unexpected adverse reaction in selected individuals. Genetic variation is a common contributor to altered responses. Pharmacogenetics, a term first coined by Friedrich Vogel in 1959 (Vogel, 1959), is the study of the association between genetic variation (most commonly single-nucleotide polymorphisms (SNPs) or microdeletions), and the efficacy and toxicity of a drug.

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2 Pharmacogenetic biomarkers

Pharmacogenetics more commonly deals with the identification of germ-line genetic variants as predictive markers of drug response. However, in some patient groups, such as those suffering from cancer, genomic analysis is possible both on germ-line DNA, often derived from blood, as well as on somatic DNA, derived from tumour tissue (biopsies or resections). Whereas germ-line DNA variants are inherited in normal Mendelian fashion, somatic DNA mutations represent the variations associated with tumour initialisation and progression, or secondary mutations due to the tumour itself (Gerlinger et al., 2012). For example, genetic variations in the genes that code for drug metabolising enzymes, such as thiopurine-S-methyltransferase (TPMT), cytochrome P450 2D6 (CYP2D6) and uridine-diphosphate glucuronosyltransferase 1A1 (UGT1A1) are germ-line polymorphisms useful for determining pharmacokinetically-influenced pharmacological outcomes. Table 1 summarises a few examples of such pharmacogenetic variability. On the other hand, acquired somatic mutations in tumour tissue may regularly directly modify the pharmacodynamics of drug response. Somatic mu-

tations are exemplified by the presence of the BRC-ABL (a reciprocal chromosomal translocation between chromosomes 9 and 22, resulting in the generation of a fusion gene, consisting of the chromosome 9 ABL1 gene and part of the BCR gene on chromosome 22) translocation in chronic myeloid leukaemia (CML), the overexpression of human Epidermal Growth Factor Receptor 2 (HER2) in breast cancer, BRAF (a gene coding for serine/threonine-protein kinase B-Raf involved in cell-growth signalling) mutations in metastatic melanoma, and Epidermal Growth Factor Receptor (EGFR) mutants in lung cancer. The abnormal functioning of these oncogenic proteins promotes cell transformation. The transformed cells become dependent on the oncogenic stimulus and hence provide a therapeutic opportunity, due to the increased sensitivity of the transformed cells to specific inhibitors. This is the basis of targeted therapies (Weinstein, 2008). The tissue-specific variations are classified as prognostic or predictive biomarkers. A prognostic biomarker is defined as a measurable disease-related parameter that is associated with clinical outcome. On the other hand, a predictive biomarker provides information on the expected response to a specific therapy, therefore identifying patients who are responsive to a specific treatment, and/or predisposed to toxicity. Interestingly, BRAF mutations provide prognostic information in colorectal cancer, but they are predictive in melanoma (Chapman et al., 2011).

3 Genetic influences on drug pharmacokinetics

The administration of a drug is followed by multiple processes, which comprise the pharmacodynamic events through which its effects manifest themselves; and pharmacokinetic events, through which the drug becomes subject to modification by physiological systems. Such pharmacokinetic events may include bioactivation to the active form, as well as various detoxification processes, which occur prior to elimination. A group of enzymes, the Cytochrome P450 (CYP450) family, are the major contributors to drug activation and metabolic processes (Guengerich, 2008; Ortiz de Montellano, 2013). CYP450 genes are highly polymorphic, resulting in variable activity of enzymes and hence variable processing of the drug within human physiology. Of interest, the polymorphic nature of these enzymes is even more pronounced across different ethnic groups (McGraw and Waller, 2012). The Human Cytochrome P450 Allele Nomenclature Committee maintain a public database, that details all known CYP450 gene alleles and the influence of these alleles on specific CYP450 enzyme activities (Human Cytochrome P450 (CYP) Allele Nomenclature Committee., 2013). Examples of CYP2D6 functional variants are discussed further below.

3.1 Biotransformation of prodrugs

The development of prodrugs provides the chemical approach to overcome the therapeutic barriers of some drugs, such as mal-absorption, inadequate distribution to specific destinations in the body and premature detoxification, which may, for example, occur during the hepatic first pass effect. Prodrugs are administered as pharmacologically inactive or low activity compounds and require prior conversion to the pharmacologically active metabolite in order to function. The exploitation of endogenous enzymatic systems for the bioactivation of prodrugs may be influenced by genetic variations within populations and ethnic groups that are associated with an increased, reduced or even no enzymatic activity.

3.1.1 Dopa decarboxylase

One of the best known pro-drug applications has been the development of L-Dopa for the management of Parkinson's Disease (PD). L-Dopa crosses the blood brain barrier, and is metabolized to dopamine in the brain by aromatic L-amino acid decarboxylase (commonly called DOPA decarboxylase, DDC). Directly administered dopamine would be unable to cross the blood brain barrier and would exert several peripheral adverse effects, making it unsuitable for management of PD. The actions of L-dopa-generated dopamine are further centralized to the brain by the co-administration of carbidopa, a peripheral dopamine decarboxylase inhibitor, which effectively minimizes peripheral metabolism of L-dopa to dopamine. Alterations in the function or expression of DDC may therefore be expected to influence L-Dopa treated PD management outcomes. Indeed, two DDC gene variants (rs921451 - an intronic T>C substitution, and rs3837091 - a four-base AGAG deletion in the non-translated exon 1) that show significant decreases in L-dopa treated PD patient management outcomes have recently been reported (Devos et al., 2014).

3.1.2 CYP2D6

Within the family of CYP450 enzymes, CYP2D6 is perhaps the most pharmacogenetically-relevant member. This is in view of its participation in the metabolic pathways of multiple drugs, originating from different pharmacological classes (e.g. selective serotonin reuptake inhibitors, opioids, tricyclic antidepressants, β-adrenergic antagonists, antipsychotics, antiarrhythmics), and the highly polymorphic nature of the gene. The enzyme is important both in lieu of its drug metabolizing and detoxification properties (discussed separately further down), as well as a participant in the metabolic activation of some prodrugs.

Table 1: A non-exhaustive list of genes, for which known alleles influence the outcomes of specific drug therapy.

Gene	Drug(s) affected	Outcome	Reference
ABCB1	Various	Variants causing ABCB1 overexpression increase cellular efflux and reduce activity of affected intracellular-acting drugs. Variants causing low ABCB1 expression tend to increase adverse effects due to low efflux and intracellular drug accumulation.	(Franke et al., 2010)
OATP1B1	rosuvastatin	OATP1B1*5 homozygotes exhibit higher plasma levels of rosuvastatin	(Choi et al., 2008)
SLCO1B1	simvastatin	Increased risk of myopathy in patients with the rs4149056 C-variant	(Brunham et al., 2012)
SLCO1B1	simvastatin	Variant rs4149056 associated with myopathy in patients on 80mg daily simvastatin	(Carr et al., 2013)
UGT1A1	irinotecan	UGT1A1*28 allele causes low drug metabolism and potential life threatening effects including myelosuppression, arrhythmia, neutropenia, thrombocytopenia and diarrhoea	(Dias et al., 2012)
G6PD	dapsone	G6PD deficient individuals may be at an increased risk of haemolytic adverse reactions	(Mason et al., 2007)
G6PD	nitrofurantoin	G6PD deficient individuals may be at an increased risk of haemolytic adverse reactions	(Youngster et al., 2010)
VKORC1	warfarin	G-1639A allele causes increased bleeding risk especially when present with CYP2C9*2 or CYP2C9*3 alleles	(Santos et al., 2013; Yang et al., 2013)
POLG	valproic acid	Increased risk of potentially fatal valproate-induced acute liver failure, especially in patients with the Q1236H substitution.	(Stewart et al., 2010)
NAT2	isoniazid, rifampicin	NAT2 slow acetylator genotypes *5, *6 and *7 are associated with higher risk of anti-tuberculosis drug-induced liver injury	(Wang et al., 2012)
CYP2C9	warfarin	CYP2C9*3 homozygotes exhibit an extended warfarin half-life with possible haemorrhagic events	(Santos et al., 2013; Yang et al., 2013)
CYP2D6	codeine	CYP2D6 ultrarapid metabolizers may show opioid toxicity due to extensive metabolism of codeine to morphine	(Kirchheiner et al., 2007)
CYP2D6	amitriptyline, nortryptiline	CYP2D6 ultrarapid metabolizers experience no response, while poor metabolizers experience drug toxicity at conventional dosages	(Teh and Bertilsson, 2012)
CYP2D6	tamoxifen	CYP2D6*10 homozygotes experience poor response to tamoxifen	(Kiyotani et al., 2012)
DDC	L-dopa	DDC rs921451 and rs3837091 variants are associated with poor L-dopa-treated Parkinson's disease management outcomes	(Devos et al., 2014)
TPMT	azathioprine	TPMT*2, TPMT*3 or TPMT*4 alleles have a low TPMT activity, and patients show azathioprine toxicity	(Ford and Berg, 2010; Relling et al., 2011)
UGT1A1	irinotecan	Variant *28 associated with high risk of neutropenia	(Perera et al., 2008)
ALOX5	ABT-761, zileuton	Patients carrying non-pentarepeat Sp1 promoter variants, express low ALOX5, and show reduced response to 5-LOX inhibitors	(Drazen et al., 1999)
ADRB2	carvedilol	CHF patients who are Gln27 homozygotes show lower response to treatment than Glu27 homozygotes or heterozygotes.	(Kaye et al., 2003)

ADRB2	salbutamol salmeterol	Variant Gly16 associated with lower response to β_2-adrenoceptor agonists	(Hall et al., 1995; Lipworth et al., 2013)
OPRM1	morphine and other opioids	A118G allele associated with reduced surface receptor expression and low response to exogenous opioids	(Zhang et al., 2005; Kroslak et al., 2007)
NR3C1	glucocorticoids	Variants Ala229Thr and Ile292Val associated with decreased receptor ligand binding affinity; variant T746C and haplotype 237delC/C238T/G240C associated with lower glucocorticoid receptor expression.	(Niu et al., 2009)
NR3C1	glucocorticoids	Glucocorticoid receptor promoter BclI G allele associated with glucocorticoid resistance	(Pietras et al., 2011)

Within the latter perspective, the CYP2D6 gene was extensively studied within the context of tamoxifen metabolism. Tamoxifen is used to treat oestrogen receptor positive breast cancer patients, and is metabolised to endoxifen, a more potent molecule, by CYP2D6. The wild type CYP2D6 (CYP2D6*1) generates an enzyme with normal activity. The variants *3, *4 and *5 have null activity and the variants *9, *10 and *17 have a decreased enzyme activity. Compared to extensive metabolisers (EMs) having both wild type alleles, patients with one null allele, or with one or more decreased activity alleles have an 'intermediate metabolism' (IM) phenotype, whilst patients carrying two null alleles are 'poor metabolisers' (PM). This variation carries therapeutic implications in patient tamoxifen sensitivity (Crews et al., 2012).

Codeine, an opiate agonist indicated for the relief of mild to moderately severe pain, is converted to an active metabolite, morphine, via the CYP2D6 enzyme, in order to exert its analgesic activity. The analgesic activity of codeine is therefore due to the combined action of the weak parent molecule and the potent CPY2D6-dependent metabolite, morphine; thus making codeine a prodrug. The presence of a CYP2D6 allele, which generate a low activity enzyme (poor metabolisers, such as individuals carrying CYP2D6*4, *5, *6, or *7 alleles) has been associated with a reduced codeine response, while high CYP2D6 activity in ultrarapid metabolisers (due to CYP2D6 gene copy number variation), have been reported to induce morphine toxicity with conventional codeine doses (Gasche et al., 2004).

CYP2D6 alleles, which mainly consist of haplotypes rather than single SNPs, have been classified by The Human Cytochrome P450 Allele Nomenclature Committee, and the specific genotype of each allele is archived at an official database hosted at http://www.cypalleles.ki.se/cyp2d6.htm.

3.1.3 Carboxylesterases

Carboxylesterases represent a class of prodrug bioactivating enzymes that are widely distributed in tissues including plasma, liver, intestine and other biological fluids (Hosokawa, 2008). They metabolize prodrugs, such as olmesartan medoxomil (an angiotensin II receptor antagonist) and oseltamivir (an antiviral agent) to their respective pharmacologically active metabolites olmesartan (Ishizuka et al., 2010) and oseltamivir carboxylate (Zhu and Markowitz, 2009). The identified carboxylesterase 1 variant Gly143Glu, has been reported to reduce the activity of the prodrug-activating enzyme to 25% of the wild type, and the Asp260fs variant ablates activity completely. This may have serious implications, with respect to the efficacy of oseltamavir in patients carrying these variants (Zhu and Markowitz, 2009).

The inhaled glucocorticoids beclomethasone and ciclesonide are also activated by lung esterases. Inhaled beclomethasone diproprionate is metabolised to the more active beclomethasone-17-monopropionate, while ciclesonide, an inactive prodrug, is metabolised in the lungs to the pharmacologically active desisobutyryl-ciclesonide. Although genetic variability in various esterase genes has been described (Wu et al., 2004; Charasson et al., 2004), pharmacogenetic evidence for their relevance to bioactivation-associated pharmacology is lacking. There is evidence, however, to indicate pharmacogenetic importance of these enzyme systems as detoxifying agents in the metabolism of active drug molecules such as methylphenidate (Zhu et al., 2008), where specific SNPs have been shown to drastically alter the rate of metabolism.

3.2 Membrane transporters

Drug transport across cell membranes may occur either by passive mechanisms, or through the mediation of specific cell-membrane proteins which actively transfer the drug molecule between the extracellular environment and the intracellular milieu. More than 400 membrane transporters are known to be coded for by the human genome, and these are differentially expressed in various tissues. Genetic variability in the two major eukaryotic transporter families, the ATP-binding cassette (ABC) and Solute Carriers (SLC), is recognised to have major influences on drug therapeutic efficacy and adverse reactions. Such changes may

be brought about by functional polymorphic variation which influences either the eukaryotic ABC-mediated efflux functions, or the SLC uptake efficiency, or both. A database of known transporters may be accessed at the Human Membrane Transporter Database, available at http://lab.digibench.net/transporter/ (Yan and Sadée, 2000).

3.2.1 ATP-binding cassette (ABC) transporter family

P-glycoprotein (Pgp)

P-glycoprotein (Pgp), a major transporter of the ABC family, is encoded for by the ABCB1 gene (previously known as MDR1) and mediates the ATP-dependent efflux of drugs from cells. It is expressed in various tissues including intestinal, hepatic and renal. It also contributes to the maintenance of the blood-brain barrier system through endothelial expression in the central nervous system. Pgp substrates originate from diverse pharmacological classes, and include immunosuppresants, cardiovascular agents, antidepressants and anti-epileptic agents, as well as cytotoxic drugs. ABCB1 overexpression is a major concern in cancer chemotherapy since it induces resistance to cytotoxic agents through increased cellular efflux. For example, high-expression inducing ABCB1 G2677T and G2995A SNPs have been identified in tumour patients, in drug-resistant cell lines (e.g. colon cancer cell lines and glioblastoma cell lines) and also in cells from refractory malignant malignomas. ABCB1-knockout mice show increased tendency toward dose-related adverse drug reactions, increased blood brain barrier transport and a degree of altered pharmacokinetics to a plethora of drugs including paclitaxel, loperamide, vinblastine, ivermectin, digoxin and cyclosporine. This is accompanied by drug accumulation in the brain, liver and intestine.

The ABCB1 C3435T SNP is associated with low intestinal Pgp expression, especially in homozygotes, and these comprise about 25% of Caucasians. The reduced intestinal cellular Pgp efflux activity in these individuals has been associated with increased plasma levels of administered digoxin, due to increased uptake of the drug from the gastrointestinal tract. There are about 20 known human ABCB1 variants to date, and their functional relevance is still coming to light. The differential expression of this gene and its recognised genetic polymorphic profile makes it an important potential target for altered pharmacokinetic behaviour of drug substrates, and the subsequent pharmacodynamic implications. For example, high Pgp activity induces cellular efflux of glucocorticoids such as prednisolone, dexamethasone and beclomethasone monopropionate (the active metabolite of the pulmonary administered beclomethasone dipropionate), thus decreasing their clinical response. There is also evidence to show that glucocorticoids upregulate Pgp expression and may therefore amplify the effects of ABCB1 allele-specific high activity variants (Crowe and Tan, 2012).

3.2.2 Solute Carrier (SLC) transporter family

Organic anion-transporting polypeptides (OATP)

Organic Anion-Transporting Polypeptides (OATP), the major SLC-type transporters, have received special attention due to their involvement in the cellular influx of several drugs, particularly statins (HMG-CoA reductase inhibitors). OATPs are highly expressed in hepatic tissue, where they are involved in the clearance of drugs from portal circulation in preparation for subsequent biliary excretion. The influx transporter OATP1B1 (coded for by the SLCO1B1 gene) has, in particular, been well studied due to its major role in the hepatic uptake of many drugs. For example, the SLCO1B1 alleles *2 (T217C), *3 (T245C, A467G), *5 (T521C), *9 (G1463C), *12 (T217C, A1964G), *13 (T245C, A467G, A2000G), *15 (A388G, T521C), and *18 (A388G, G1463C) have all been associated with decreased rosuvastatin transport activity. SLCO1B1*15 homozygotes in particular have been reported to exhibit significantly higher AUC values for concentration-time curves of plasma rosuvastatin, possibly due to decreased intracellular drug influx (Choi et al., 2008).

Na^+-taurocholate cotransporting polypeptide (NTCP)

A variant of another SLC member, the Na^+-taurocholate cotransporting polypeptide *2 allele (NTCP *2, C800T), is known to have a near complete loss of function for bile acids; however it exhibits a profound gain of function for rosuvastatin, resulting in a clinically relevant reduction in plasma levels compared to the wild-type allele. The outcome may be further complicated by the contribution of OATPs such as OATP1B3, OATP2B1 and OATP1A2, which are also known to mediate statin transport and exhibit functional polymorphic variation in humans (Choi et al., 2011).

Organic anion transporter family member 1B1 (SLCO1B1)

The solute carrier organic anion transporter family member 1B1 (coded for by SLCO1B1) is expressed and localised in the cell membrane of hepatocytes and is involved in uptake of xenobiotic and endogenous substances (Karlgren et al., 2012). During the recent years, the SLCO1B1 SNP T37041C (rs4149056) began to receive special attention following the development of myopathy in patients on high dose simvastatin (80mg daily) participating in the 2008 SEARCH case-control study, and who were later recognised to carry the minor

C allele. The localisation of the transporter in the hepatic plasma membrane is disturbed in the presence of this variation, resulting in a higher simvastatin plasma concentration and increased skeletal muscle drug exposure (Pasanen et al., 2006). The FDA strongly advises against 80mg daily simvastatin doses for patients who are heterozygous or homozygous for the C allele. The FDA also advises caution for long term use of 80mg daily doses in wild type 37041T homozygotes. These recommendations have also been incorporated into the SLCO1B1 Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines (Wilke et al., 2012).

3.3 Drug metabolism

Drug metabolism is a complex process, often involving multiple parallel pathways, during which drugs are converted to breakdown products (usually of lower toxicity) in preparation for elimination from the physiological system. The rate of drug breakdown is important in reducing toxicity level and maintaining a pharmacological dose within the therapeutic window of the active ingredients.

3.4 Thiopurinemethyltransferase (TPMT)

Thiopurinemethyltransferase (TPMT) is a phase II biotransformation cytosolic enzyme catalysing the methylation of thiopurines into inactive metabolites. The TPMT enzyme is highly expressed in the liver, while its expression is low in the brain and lung (Ford and Berg, 2010). Interestingly, the TPMT activity in erythrocytes correlates with the hepatic enzyme activity, meaning that phenotypic assessment can be performed in erythrocyte lysates (Wu, 2011). The TPMT gene is polymorphic and despite ethnic variability, the minor alleles TPMT*2, TPMT*3A and TPMT*3C account for the majority of the low-activity variants (Relling et al., 2011). TPMT activity shows a trimodal distribution with heterozygotes having intermediate activity (IM), homozygotes or compound heterozygosity showing low or absent activity (PM), and the normal genotype TPMT*1/*1 representing the normal activity of the enzyme (EM). The major drug for which TPMT activity is relevant is mercaptopurine, the active metabolite of the prodrug azathioprine. Mercaptopurine is detoxificated by several metabolic pathways, including a major one that involves the enzyme TPMT. Azathioprine patients with low TPMT activity are at a strong risk of developing severe, potentially life-threatening, bone marrow toxicity when treated with conventional doses of azathioprine or mercaptopurine (Anon, 2009; Ford and Berg, 2010).

3.5 UDP Glucuronosyltransferase 1 (UGT1A1)

Irinotecan, a topoisomerase I inhibitor used to treat several solid tumour types, as well as its active metabolite SN-38, exert their effects by preventing re-ligation of single-stranded DNA breaks induced during the DNA synthesis phase of cellular replication. SN-38 subsequently undergoes glucuronidation primarily in the liver by UGT1A, followed by excretion through the kidneys.

Adverse effects of irinotecan treatment include severe diarrhoea, myelosuppression, and neutropenia. These effects are induced by inefficient UGT1A1-dependent metabolism of SN 38 (Mathijssen et al., 2001). UGT1A1 gene variants, which demonstrate a reduced enzyme activity or expression, cause an accumulation of the active metabolite SN-38 at conventional urinotecan dosing. Of primary importance is the high frequency variant UGT1A1*28, which results in at least 70% reduction in expression levels (Perera et al., 2008). Cancer patients homozygous for the *28 allele, receiving irinotecan at 350 mg/m^2 every 3 weeks, have a high risk of suffering from neutropenia. Genotype-guided phase I studies determined the maximum tolerated dose in *1/*1, *1/*28, and *28/*28 patients to be 390, 340, and 150 mg/m^2, respectively (Marcuello et al., 2011). Indeed, the FDA recommends an initial dose reduction of irinotecan for patients who are homozygous for UGT1A1*28, this is supported by the CPIC and Dutch Pharmacogenetics Working Group (DPWG) guidelines, which recommend a 30% dose reduction in irinotecan administration for this genotype (Relling and Klein, 2011).

3.6 Uridine diphosphate glucuronosyltransferase 2B7 (UGT2B7)

The UGT2B7 gene is highly expressed in the liver, and is pharmacogenetically relevant due to its importance in the detoxification of morphine. The gene product converts the drug to the inactive form morphine-3-glucuronide and the low efficacy morphine-6-glucuronide metabolites. Two tightly linked UGT2B7A polymorphisms, the 161C/T promoter SNP and the 802C/T SNP, are associated with low glucuronidation of morphine, with consequent clinical implications in opioid efficacy (Sawyer et al., 2003; Eissing et al., 2012).

3.7 Cytochrome P450 enzyme system (CYP450)

Some examples involving CYP450 members concerned in prodrug activation have already been cited earlier in this article. However, the metabolic detoxification role of these enzymes has more widespread implications, and has been much more thoroughly studied. Indeed, this enzyme system has been reported to partake in over 75% of drug detoxification processes that occur in man.

CYP2D6

The CYP450 member, which is most relevant to drug metabolism processes is the D6 enzyme of the CYP450 family 2 (CYP2D6). This enzyme is capable of metabolizing drug substrates that originate from chemically and pharmacologically distinct groups, including antidepressants, antipsychotics, antiarrhythmic, anti-emetics, beta-blockers and opioids. It is estimated that about 25% of currently marketed drugs have their metabolism in some way influenced by CYP2D6 activity, with about 100 commonly used drugs being major substrates for the enzyme.

The CYP2D6 gene is highly polymorphic. The Human Cytochrome P450 (CYP) Allele Nomenclature Committee currently lists over 100 known CYP2D6 genotypes, which may result in the expression of a normal or altered-activity CYP2D6 enzyme (http://www.cypalleles.ki.se/cyp2d6.htm). According to the specific allele combination carried, individuals may be classified as poor, intermediate or extensive metabolisers. Moreover, copy number variations of the CYP2D6 gene have also been described in the literature (Sheng et al., 2007), leading to the ultrarapid metaboliser phenotype. It has been estimated that as much as twenty million inhabitants of Western Europe are ultrarapid metabolisers, with at least a copy number of 2 functional CYP2D6 alleles (Ingelman-Sundberg, 2005). Extensive and ultrarapid metabolisers may show insufficient or no clinical response to CYP2D6 enzyme substrate drugs used at standard conventional dosages, while poor metabolisers run the risk of drug accumulation carrying consequent adverse reactions. For example, adverse effects to metoprolol, nortryptiline, perphenazine, thioridazine, haloperidol and perhexiline have been reported to be much more common in poor CYP2D6 enzyme metabolisers, while extensive metabolisers tend to show reduced effects (Teh and Bertilsson, 2012). Post-menopausal oestrogen receptor positive breast cancer patients on tamoxifen, who are also CYP2D6*4 homozygotes, show a significantly reduced relapse-free survival period when compared to wild type patients. Studies in Asian and Dutch populations have reported similar low tamoxifen response in breast cancer patients who are CYP2D6*10 homozygotes. It has been suggested that an increase of 50 to 100% of the normal tamoxifen dose is required for patients who are heterozygous or homozygous for the CYP2D6*10 allele, in order to maintain normal blood levels (Kiyotani et al., 2012).

The importance of CYP2D6 pharmacogenetics is further accentuated by the fact that the allelic frequencies of this gene are well known to exhibit diverse ethnic discrepancies, and alleles which may be present in one population or ethnic group, may be completely absent in another. This adds complexity to the genotype-phenotype interpretations.

CYP2C9

CYP2C9 gene polymorphic variability has been reported to be of pharmacogenetic relevance in the metabolism of warfarin (a commonly used anticoagulant) and celecoxib (a cyclooxygenase 2-specific non-steroidal anti-inflammatory drug). The nonsynonymous polymorphisms CYP2C9*2 (rs1799853) and CYP2C9*3 (rs1057910) express a reduced activity variant of the enzyme. CYP2C9*3 homozygotes may exhibit a warfarin half-life of 90-200 hours, thus potentially leading to serious warfarin-induced haemorrhagic events (Jorgensen et al., 2012). The same CYP2C9*3 genotype also increases the half-life of celecoxib by about 2.7 fold, and *3 homozygotes therefore warrant lower doses of the drug in order to avoid dose-related adverse effects (Prieto-Pérez et al., 2013).

4 Genetic influences on drug pharmacodynamics

4.1 Cell signalling

About seventy percent of drugs licensed for human therapeutic use mediate their action through their interaction with one or more cellular receptor proteins. Two thirds of these are dependent on one or more members of the G-protein coupled receptor superfamily. The rest act via nuclear or kinase receptors. Drug receptors, therefore, often constitute the first interface between a therapeutically administered drug and cellular physiology. Genetic variability in the receptors themselves, or any proteins involved in receptor signalling pathways, may exert a potential influence on therapeutic outcomes.

4.1.1 G-protein coupled receptors (GPCRs)

Genome sequencing data suggests that the human genome codes for about 800 different GPCRs. Nearly 400 of these are currently indexed in the International Union of Basic and Clinical Pharmacology online database (IUPHAR database, 2013), together with additional data such as: known ligands, gene and protein records and signalling pathways. The intricate signalling and trafficking pathways of GPCRs provide several targets within which genetic variability may influence drug efficacy, potency and safety. Besides the commonly reported ligand binding and subsequent signalling pathways, GPCRs may also show constitutive activity and may undergo other processes such as: desensitization, supersensitization, downregulation, upregulation, homodimerization, heterodimerization, trafficking and recycling (Bosier and Hermans, 2007;

Thompson et al., 2008b; Thompson et al., 2008a).

The most common loci that are known to cause perturbation of GPCR function however, remain to be those within the actual receptor proteins and G-protein complexes, as well as their respective gene promoters. We present some examples of pharmacogenetically relevant GPCR variants.

β_2-adrenoceptor

One of the most pharmacogenetically studied GPCRs is the β_2-adrenoceptor, coded for by the ADRB2 gene located at 5q31-q32. Polymorphic variations in this gene have been associated with: (i) perturbations of molecular pharmacological actions, such as alterations in downregulation activity, (ii) changes in therapeutic outcomes of β_2-adrenoceptor agonist treatment and (iii) changes in clinical manifestations of different diseases. For example, the widely studied Arg16Gly receptor variant displays enhanced agonist-promoted downregulation, while the Gln27Glu polymorphism appears to confer resistance to downregulation. Patients with asthma carrying the Arg16Gly β_2-adrenoceptor variant, have been reported by some research groups to exhibit a lower lung function than Arg16 patients, and also show an increased incidence of familial nocturnal asthma. These patients have also been reported to be less responsive to the commonly used β_2-adrenoceptor agonist bronchodilator treatment, probably due to reduced cell surface density of β_2-adrenoceptors. However, other clinical studies in asthmatic patients have reported ADRB2 genotype-phenotype associations in small-scale adult clinical studies, that conflict with earlier findings (Bleecker et al., 2007), and research workers have argued that sample size, patient selection, and concomitant treatment could confound the study outcomes. (Hall et al., 1995; Tan et al., 1997; D'Amato et al., 1998; Lee et al., 2004; Tattersfield and Hall, 2004). Recently, (Lipworth et al., 2013) studied 62 persistent asthmatic children who were homozygous for the ADRB2 Arg16 polymorphism and were being treated with regular inhaled fluticasone. In these Arg16 homozygotes, add-on therapy, with the leukotriene receptor antagonist montelukast, produced significantly greater clinical improvements than the long acting β_2 adrenoceptor agonist salmeterol when assessed after one year. The authors suggest that these preliminary data indicate that genotyping of the ADRB2 codon 16 may be a useful pharmacogenetic marker, which could have potential to be used to optimize asthma management (Lipworth et al., 2013; Sayers, 2013).

Leukotriene receptors

Leukotrienes constitute an important group of pro-inflammatory and bronchoconstrictory molecules, derived via the 5-lipoxygenase-mediated (5-LOX) arachidonic acid metabolism pathway. The major cysteinyl leukotrienes comprise LTC4, LTD4 and LTE4, while LTB4 which lacks cysteine, is classified separately. The former exert their actions via agonism at the the cysteinyl leukotriene receptor CysLTR1 (and to a lesser degree via CysLTR2), while the latter exerts its actions via LTB4R and LTB4R2. Antagonism of the CysLTR1 receptor by drugs such as zafirkulast, montelukast and pranlukast is an important therapeutic modality in the management of asthma and allergic rhinitis. Although genetic CysLTR1 variability is well documented, functional pharmacological effects have mainly been observed when CYSLTR1 SNPs are present together with SNPs in the 5-lipoxygenase gene ALOX5. For example, ALOX5 promoter variants, involving polymorphisms which result in an alteration of the number of Sp1 binding motifs, from the wild-type penta-Sp1 repeat, are known to result in low 5-lipoxygenase expression. This results in a reduced contribution of the specific 5-LOX pathway to overall airway inflammatory pathology, consequently reducing the clinical efficacy of both 5-LOX enzyme inhibitors as well as CysLTR1 receptor inhibitors (Kalayci et al., 2006). The CysLTR1 G300S variant confers a stronger potency to the potent bronchoconstrictor LTD4, and may confer low response to CysLTR1 receptor antagonists at conventional doses (Thompson et al., 2007; Duroudier et al., 2009)

Opioid receptors

Opioids exert their actions through their agonistic properties on three major classical GPCRs, namely the μ-receptor coded by the OPRM1 gene at 6q24-q25, the κ-receptor coded by OPRK1 at 8q11.2 and the δ-receptor coded by OPRD1 and located at 1p36.1-p34.3. These receptors are differentially expressed, exist as different subtypes, and mediate the various opioid effects such as analgesia, euphoria, dysphoria and dependence. A fourth receptor, orphanin FQ, encoded by the OPRL1 gene located at 20q13.33, is also known to be activated by opioid ligands.

The A118G, (rs1799971, allelic frequency=0.19) SNP in the OPRM1 gene has probably been the most studied. Molecular work suggests that the G allele results in a gain of function of the μ-receptor to the endogenous opioid β-endorphin, but a loss of function to exogenously administered opioids such as morphine. This has been corroborated with clinical evidence, where the G allele has been associated with a reduced morphine response in cancer and post-operative patients. However, in a meta-analysis of opioid pain studies, the authors could only detect weak associations with increased morphine dose requirements in homozygous carriers of the variant G allele. This suggests that clinical opioid response

may be the result of complex interactions between various pharmacogenetic variants. It could also be due to other common functional variants which have not been studied during meta-analysis and could therefore confound the outcome. (Mura et al., 2013; Crist and Berrettini, 2013). For example, *in vitro* studies suggest that another common variant, the exonic OPRM1 C17T, may be a relevant marker of low buprenorphine efficacy in opioid dependence management therapy (Bajada, 2010). Moreover, opioid response is known to also be influenced by other genetic polymorphisms, besides those affecting the receptors themselves. Such genes include those that code for metabolic enzymes such as CYP2D6, CYP3A4, CYP3A5, UGT2B7 and membrane transporters such as ABCB1.

4.1.2 Nuclear receptors

The nuclear receptor family, although much smaller than the GPCR group, comprises important targets for pharmacological management.

Glucocorticoid receptor

The human glucocorticoid receptor (hGR), a 94-kDa protein encoded by the NR3C1 gene located at 5q31-q32, has received significant pharmacogenetic attention, mainly due to the wide array of glucocorticoid agonists available in the therapeutic repertoire. Glucocorticoid responses are the fruit of complex multiple pathways, involving the activation or repression of multiple genes, and genotype-phenotype associations, are therefore often very difficult to establish. Moreover, although the human genome only contains one known glucocorticoid receptor gene, there are several receptor isoforms which are the result of polymorphisms, alternative splicing and alternative translational initiation. Furthermore, hGR isoforms may also be subject to a variety of posttranslational modifications, resulting in altered receptor function.

Within a sample of 240 individuals, Niu and co-workers identified 108 polymorphisms present in a range of exonic, intronic and untranslated regions of the NR3C1 gene. Subsequent functional analysis of a candidate subset of these variations identified some of these regions to be associated with higher hGRα protein expression (Phe(65)Val and Asp(687)Glu), some associated with decreased ligand binding affinity (Ala(229)Thr and Ile(292)Val) and others to be associated with decreased hGRα transcript expression (746T>C and haplotype 237delC / 238C>T / 240G>C). These functional polymorphisms were present in allelic frequencies in the range of 5.8% and 18.3% in different ethnic groups, and could contribute to reduced glucocorticoid sensitivity in a clinical setting (Niu et al., 2009).

The major issues associated with glucocorticoid therapy relate to the potential adverse effects (e.g. hypothalamic-pituitary-adrenal axis suppression, development of cushingoid features, hyperglycaemia, easy bruising, immunodeficiency) and to a patient subset who wish to refrain from this treatment. Both variables may not necessarily be directly associated to allelic variation within the NR3C1 gene, but may also be the result of downstream effectors which are responsive to hGR-induced pathways, or metabolic pathways which modify the pharmacokinetics of these drugs. For example, allelic variations in 8 genes (CNTNAP2, LEPR, CRHR1, NTAN1, SLC12A3, ALPL, BGLAP, APOB) have been associated with prednisolone-induced hypertension following a 28-day treatment remission induction of acute lymphoblastic leukaemia in children (Kamdem et al., 2008). Most of these genes are involved in the hypothalamic-pituitary axis pathway. Furthermore, as discussed earlier, gain of function polymorphisms in the ABCB1 gene, coding for the Pgp efflux transporter, have also been reported to reduce glucocorticoid efficacy by actively lowering intracellular glucocorticoid concentrations.

The main cellular hGR signalling activity is attributed to the ubiquitly expressed wild type isoform, hGRα. The β-isoform, a shortened splice variant of NR3C1, is also ubiquitly expressed, though at a lower level than hGRα. High expression levels of hGRβ are strongly associated with glucocorticoid resistance. The mechanism of this has been subject to many debates, especially since in contrast to hGRα, the β-isoform interacts poorly with heat shock proteins, does not bind ligands, and is transcriptionally inactive. The postulated mechanisms have included competition for hGRα binding sites (glucocorticoid responsive elements) on gene promoters, direct inactivation of the hGRα isoform by heterodimerization, and the inhibition of co-activating proteins which are necessary for hGRα activity. More recent evidence suggests that hGRβ competes with hGRα for binding to glucocorticoid receptor-interacting protein 1 (also called nuclear receptor coactivator 2 and coded for by the NCOA2 gene), thus generating an ineffective co-activator complex. Furthermore, a recent paper (Vazquz-Tello et al., 2013) has reported evidence showing that the cytokines IL-17 and IL-23, released by infiltrating T-cells in asthma, may contribute to hGRβ upregulation and consequently but only to a degree also contribute to glucocorticoid insensitivity.

The differential expression of the various hGR isoforms is driven by the selective use of at least 5 recognised hGR promoters (Russcher et al., 2007). The activity of these transcripts is further dependent on cell types and on promoter polymorphic variation. For example, the G allele of the BclI hGR promoter poly-

morphism is significantly associated with GC resistance and with the development of severe asthma (Pietras et al., 2011). The -22C>A polymorphism, located upstream of the hGR gene, causes a significantly lower transcriptional activity compared to the wild type C allele, as determined by promoter luciferase reporter assays in HepG2 and HEK293 cells, and is likely to be related to lower hGRα expression in the clinical setting.

The elucidation of clear hGR-associated pharmacogenetic genotype-phenotype relations remains a challenge. The complex molecular network involved in the outcomes of glucocorticoid receptor activation, suggests that the search for glucocorticoid pharmacogenetic variants should be spread over a large gene subset. Moreover, the effects of polymorphisms on the interrelated functions of these gene networks might be more important than the genetic variability present in the glucocorticoid-interacting receptor alone.

4.1.3 Enzyme pathways

The arachidonic acid cascade

Arachidonic acid is a polyunsaturated fatty acid present in the phospholipids of cell membranes. It is released from phospholipid molecules through the actions of phospholipase A2 (PLA2) and is subsequently metabolized by a variety of pathways to products which are normally necessary for cell function and development, but this may cause pathologic manifestations if overproduced. The cycloxygenase (COX) pathway, mainly driven by the enzymes COX1 (coded for by PTGS1) and COX2 (coded for by PTGS2), in particular, is a target for a wide array of non-steroidal anti-inflammatory drugs (NSAIDs), which act to inhibit their activity. The PTGS1 gene contains two strongly linked SNPs (A842G and C50T) which when present in heterozygous patients, have been shown to significantly increase the susceptibility of COX1 for inhibition by aspirin. This carries the risk of increased aspirin toxicity at conventional doses, since many NSAID adverse effects are related to over suppression of COX activity. For example, NSAID-induced peptic ulceration is associated with over suppression of COX-mediated prostaglandin E2 (PGE2) production, and high anti-platelet effect is associated with excessive inhibition of COX-mediated thromboxane A2 (TXA2). On the other hand, several COX polymorphisms (PTGS1: C22T, C50T, A842G, G128A, C644A, C714A, C10427A, G1446A; PTGS2: G765C) have been reported to cause resistance to the pharmacological effects of aspirin (Xu et al., 2012). The arachidonic acid pathway is not the sole source of NSAID-related pharmacogenetic variability. For example, lumiracoxib, (a COX2-selective NSAID) hepatotoxicity has been strongly associated with the human leukocyte antigen haplotype HLA-DRB1*1501-HLA-DQB1*0602-HLA-DRB5*0101-HLA-DQA1*0102 (Singer et al., 2010).

The lipoxygenase pathway

The enzyme 5-lipoxygenase (5-LOX) metabolizes arachidonic acid to a family of leukotrienes, amongst which the most important are LTB4, LTC4 and LTD4. All of these exhibit bronchoconstrictory and mucosecretory properties within the respiratory tract, and have been shown to be important mediators of airway inflammatory diseases, such as asthma and allergic rhinitis. 5-lipoxygenase is coded for by the ALOX5 gene located at 10q11.2, and the enzyme is activated by the 5-lipoxygenase activating protein (ALOX5AP gene located at 13q12). Transcriptional regulation of ALOX5 is heavily dependent on a pentarepeat SP-1 binding motif repeat located in its promoter. Lower or higher numbers of ALOX5 promoter SP-1 repeats have been shown to significantly reduce ALOX5 gene expression. Indeed, asthma patients carrying a variant SP-1 repeat number show minimal or no improvement following treatment with zileuton, a 5-LOX inhibitor, due to the reduced expression of the drug target. The functional outcome of this polymorphism can be compounded further by concomitant polymorphic variation in other pathway-related genes, such as 5-lipoxygenase activating protein (coded for by ALOX5AP), leukotriene A4 hydrolase (coded for by LTA4H), leukotriene C4 synthase (coded for by LTC4S), and the leukotriene receptors CYSLTR1 and CYSLTR2. (In et al., 1999; Drazen et al., 1999; Telleria et al., 2008; Geiger et al., 2009; Duroudier et al., 2009; Tantisira and Drazen, 2009). Interestingly, the ALOX5 haplotype [-1708G]-[21C]-[270G]-[1728A] appears to contribute to aspirin sensitivity in asthma, and may potentially help to identify aspirin-sensitive from aspirin-tolerant asthmatic patients (Choi et al., 2004).

Due to the common arachidonate substrate of the COX and 5-LOX enzymatic pathways, events occurring within one pathway can also influence the other. For example, COX-inhibition by NSAIDS enables the unmetabolized arachidonic acid to shunt to the 5-LOX pathway, thus generating higher amounts of bronchoconstrictor leukotriene products. This has been the basis of the bronconstrictory adverse effect of NSAIDs often seen in aspirin-intolerant asthmatics; an effect which is exacerbated by the presence of genetic variants which influence the 5-LOX arm of arachidonate metabolism. The first reported such polymorphism was the leukotriene C4 syntase (LTC4S) promoter SNP (-444 A/C), which was associated with aspirin-induced asthma exacerbation, potentially due to LTC4S-mediated increased leukotriene production in carriers of this allele (Sanak et al., 1997). This association, which was derived from a small adult patient-based

study, has unfortunately not been replicated by other studies. In addition, luciferase-based promoter reporter experiments in HeLa cells and KU812F cells have not been able to identify any influence of these polymorphisms on promoter transcriptional activity. However, this SNP does seem to contribute to lower pulmonary function FEV1 readings in children (Sayers et al., 2003).

Tyrosine kinases

Tyrosine kinases constitute a family of enzymes that owe their importance to their tight involvement in proliferative cell signalling and cancer management. Kinase variants provide a robust way to enable the classification of patients suffering from several types of cancers, into specific therapeutic groups, based on pharmacogenetically-based predictive therapy outcome. Genotyping of tyrosine kinase variants has applications in predicting therapy efficacy as well as drug resistance. This may be exemplified by the characterisation of the BRC/ABL translocation in Chronic Myeloid Leukaemia (CML). The development of imatinib, a small molecule tyrosine kinase inhibitor, binding specifically to the ATP pocket of the BCR/ABL kinase domain, is intended for use in BCR/ABL positive leukaemia.

The characterization of the molecular mechanism of disease thus allows for the development of patient-targeted therapy. Targeted therapy has been implemented in the clinic and includes the use of the monoclonal antibodies rituximab and trastuzumab to target CD20 positive lymphomas and HER2 positive breast cancer cases respectively. Small molecule inhibitors, such as imatinib, were designed to specifically inhibit the tyrosine kinase fusion protein bcr/abl in CML. In addition, imatinib inhibits the receptor tyrosine kinase, cKIT and hence targets activation of cKIT mutants in Gastrointestinal Stromal Tumours (GIST). Table 2 lists current therapies used to target specific genetic aberrations.

Table 2: Examples of genetic variants that are used to enable optimum selection of specific oncology therapy. These biomarkers, which may be identified by appropriate genotyping, can predict whether the drug will show an adequate response or whether the patient will be resistant to its actions. The FDA advocates in favour of predictive genotyping for the intended use of these drugs for targeted therapy.

Drug	**Drug Target**	**Disease**	**Resistance to Therapy**	**Reference**
imatinib	BCR-ABL	Chronic Myeloid Leukaemia	BCR-ABL mutations	(Gorre et al., 2001; Zhang et al., 2009)
imatinib	cKit receptor	Gastrointestinal Stromal Tumours	KIT V654A	(Rubin et al., 2010; Chen et al., 2004)
trastuzumab pertuzumab	HER2 receptor	Breast Cancer	PIK3CA mutants	(Kataoka et al., 2010)
vemurafenib	BRAF V600E	Metastatic Melanoma	RAS mutations (activation of MAPK)	(Chapman et al., 2011)
erlotinib	EGFR L858R; Δexon 19	Non-Small-Cell Lung Carcinoma	EGFR (T790M)	(Miller et al., 2012; Kwak et al., 2010)
cetuximab	EGFR	Colorectal Cancer	KRAS mutations	(Liévre et al., 2006; Allegra et al., 2009)

Activating tyrosine kinase mutations are central to specific targeted therapy. Investigation of kinase deregulation within particular patient groups, has led to identification of mutant tyrosine kinases associated with disease progression and therapy modulation. Of interest is the identification of mutations in the receptor tyrosine kinase cKit, associated with high relapse risk in core binding factor leukaemias (Paschka et al., 2006). Mutations in exon 8 and 17 of the KIT gene significantly decrease the overall survival of acute myeloid leukaemia patients with the translocations inv(16) and t(8;21). The potential specific targeting of cKit using small molecule tyrosine kinase inhibitors necessitates the promotion of screening patients with core-binding factor AML for KIT mutations. This genetic profile of specific groups of patients gives prognostic and predictive information of clinical relevance (Cammenga et al., 2005).

In addition to high relapse rate, therapy outcome markers include mutations that predict resistance to therapy. In colorectal cancer (CRC) treatment, K-Ras mutations significantly predict resistance to anti-EGFR therapy. Several clinical studies have shown that the presence of a K-Ras mutation is a significant predictor of resistance to anti-EGFR therapy in colorectal cancer patients (Liévre et al., 2006). The identification of K-Ras mutants in CRC became part of the clinical practice protocols and clinical trials. Table 2 presents a list of mutations that are increasingly being used to pre-

dict therapy outcome. There are various mechanisms of resistance that account for primary and secondary (acquired) lack of therapy response. Point mutations in the kinase domain of the target molecule conferring steric hindrance account for the majority of primary resistance.

5 Analytical platforms for pharmacogenetics

For several years, genotyping technology has only been accessible through the expertise and hardware platforms of specialized laboratories. There is now a gradual movement to bring pharmacogenetic genotyping technology to the bedside, through portable automated equipment, which transfers most of the onus of obtaining good analytical quality data to the instrument, rather than the operator. An example of this is the patented FDA-approved Spartan RX CYP2C19 test, which analyses *2, *3, and *17 alleles, and is specifically relevant to CYP2C19-metabolized substrates, such as the antiplatelet drug, clopidogrel. The portable genotyping device uses a buccal swab as the analyte and delivers genotyping results within 60 minutes, with minimal operator intervention.

Larger scale genotyping assays remain within the domain of specialized centres. For example, the Affymetrix DMET+ microarray chip is capable of simultaneously analysing a panel of 1936 pharmacogenetically-relevant mutations from 225 different genes consisting of 50 CYP450 genes, 45 Phase II enzymes, 64 drug transporters and 66 transcriptional regulators and enzymes. The Affymetrix Amplichip, marketed by Roche, uses similar microarray technology applied on a smaller scale, to provide genotype data of a panel of CYP2D6 and CYP2C19 polymorphisms which are relevant to the metaboliser status for specific β-adrenergic receptor inhibitors, antidepressants, anti-psychotics, proton-pump inhibitors, anti-epileptics and opioids.

It is the challenge of scientists and technology to place more pharmacogenotyping tools in the hands of drug prescribers, to further enhance reaping the benefits of personalized medicine.

6 Conclusion

Over one hundred drugs currently approved by the FDA, require the inclusion of pharmacogenomic information on the drug label (FDA, 2013). This translates to approximately 25% of patients who make use of hospital outpatients services, being prescribed one or more drugs that have pharmacogenomic information on the labelling (Frueh et al., 2008). For these therapeutic groups, clinically relevant information obtained using pharmacogenetic biomarkers is a warranted approach to allow proper classification of patients into predictive therapeutic categories. Integrating this information into patient management protocols allows selection of personalized targeted treatment regimes, reduces interpatient drug response heterogeneity, saves unnecessary drug toxicity and decreases morbidity.

In order to accomplish this target, multiple approaches need to be conducted in parallel. Pharmacogenetic data must be validated through repeatability studies and cumulated into curated repositories. Indeed, such repositories have rapidly developed in the last 10 years (Patrinos and Brookes, 2005; Lagoumintzis et al., 2010). Besides the "Table of Pharmacogenomic Biomarkers in Drug Labeling" provided by the FDA (FDA, 2013), other noteworthy repositories are the PharmGKB Pharmacogenomics Knowledgebase, managed by the PharmGKB team at Stanford University, California, USA (Whirl-Carrilo et al., 2012) (Pharmacogenomics Knowledgebase (PharmGKB), 2013) and FINDbase, maintained by the GoldenHelix Institute of Biomedical Research, London, UK (Georgitsi et al., 2011) (FINDbase Genome Variation Allele Frequencies Worldwide, 2013).

Actions toward translational pharmacogenetics are needed (Squassina et al., 2010). Indeed, large scale initiatives to consolidate pharmacogenomic data and translation of these from bench to bedside are currently underway. The Pharmacogenomics for Everyone (PGENI) initiative aims to focus pharmacogenetic data into a country-specific knowledgebase in order to augment the relevance and applicability for individual patients (Pharmacogenomics for Everyone Initiative (PGENI), 2013). The Clinical Pharmacogenetics Implementation Consortium (CPIC), established in 2009, is a shared project between PharmGKB and the Pharmacogenomics Research Network. CPIC publishes peer-reviewed guidelines to pharmacogenetic-guided drug dosing, and simultaneously posts these recommendations to the online curated PharmGKB repository (Clinical Pharmacogenetics Implementation Consortium (CPIC), 2013).

Pharmacogenetic stratification of patients in clinical trials opens a new door to enable early identification of specific drug responses in a genetically defined patient subgroup. The inclusion of such stratification, from drug development Phase II clinical trials onwards, generates drug outcome data that may be otherwise genetically confounded and therefore not statistically identifiable. This is especially relevant for diseases such as asthma, for which therapies directed at new drug targets are currently under development (Portelli and Sayers, 2012). This approach has to be combined with the use of new large scale high throughput omics-based technologies, in order to identify novel functional genetic

loci, which either on their own or in combination, can be used as predictive biomarker panels.

Pharmaceutical and diagnostic companies are working together to formulate robust pharmacogenetic tests which could be used to predict the best drug at the right dose for a specific patient. This goal aims to achieve safer and more effective patient outcomes and in the long term, decrease pharmacoeconomic-related costs. Various issues including complex genotype-phenotype associations, variable penetrance, gene-gene interactions, and ethnic-dependent differences in allelic distributions, make this a very challenging task. Its success depends entirely on the concerted efforts of industry, academia and regulatory agencies, combined with the provision of relevant educational services for drug prescribers and associated healthcare providers.

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