Pharmacokinetics and Pharmacogenetics: Bringing the Magic Bullet Closer to Reality

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Abstract Why do some patients respond positively to some drugs, while others may experience adverse effects? Can we predict which patients will react in which way? Does a magic bullet exist? The trend towards pharmacogenetics and personalised medicine in the last few years has somewhat sidelined the relevance of the traditional pharmaceutical sciences, such as pharmacokinetics and pharmacodynamics. Yet these actually are part and parcel of pharmacogenetics. Indeed understanding pharmacokinetics and pharmacodynamics in pharmacogenetics is essential in assessing the risk of new chemical entities (NCEs) in populations and individuals. Clinical pharmacokinetics, in fact, can be understood to have been a precursor to the implementation of pharmacogenetic understanding in the clinical setting. In this chapter, examples will be given of the strong interrelation between pharmacogenetics and the various pharmacokinetics processes i.e. absorption, distribution, metabolism and elimination. Reference will be made to studies which have shown how pharmacogenetics can be reinterpreted into pharmacokinetic principles, thus leading to the individualisation of drug therapy in the individual patient. The impact on recent regulatory guidelines published on the role of pharmacokinetics in pharmacogenetics and their impact on regulation of new medicinal drug development will also be discussed.

Keywords Pharmacokinetics · Pharmacodynamics · Pharmacogenetics · ADME · Drug transporters · Therapeutic drug monitoring · Individualised therapy · Drug development

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1 Introduction: What is PK and What is its Role in PGx?

Drugs are, fundamentally, chemical compounds and drug therapy is a dynamic process. Thus the body handles the drug as it would any other chemical compound as it moves in the body. This fate of the drug proceeds over a certain time interval and the various, so called 'pharmacokinetic' processes (from Ancient Greek *pharmakon* "drug" and *kinetikos* "to do with motion") determine the time course of the drugs: drugs are *liberated* from the formulation; *absorbed* through the administration site; *distributed* through the body; *metabolised* mostly in the liver (but not only); *eliminated* mostly in the kidneys (but not only), often given the acronym of ADME [1].

Pharmacokinetics was first conceived as a term in 1950s by the German Professor Dost. Coincidently it was around the same time that Vogel Friedrich in 1959 published his key paper *Moderne probleme der Humangenetik*—the influence of genetic factors on the response to drug [2].

Pharmacokinetics was popularised by Holford in 1982 with his use of the aphorism 'pharmacokinetics is what the body does to the drug while pharmacodynamics is what the drug does to the body' [3]. Pharmacokinetic/pharmacodynamic (PK/PD) relationships are important in the drug therapy because they are predictive sciences (Fig. 1). They are essential to determine drug plasma/response relationships and thus the dose and dosage regimen needed in an individual patient, predict drug-drug and drug-food interactions and, in fact, form the basis of what has become more colloquially as *personalised medic*ine. It is mandatory for regulatory purposes that new chemical entities (NCEs) in various stages of drug development have their pharmacokinetic parameters well characterised with the use of software such as WINNONLIN® and NONMEM®, prior to marketing authorisation.

The *FDA Critical Path Initiative* and *NIH Roadmap* in 2004 changed the focus in innovation in drug development towards one based on translational science. It also led to a renewed understanding of the importance of PK/PD as quantitative pharmacology and led to terms such as "pharmacometrics", and "model-based drug development" [4]. This led to a shift from traditional paradigms, such as the ef-

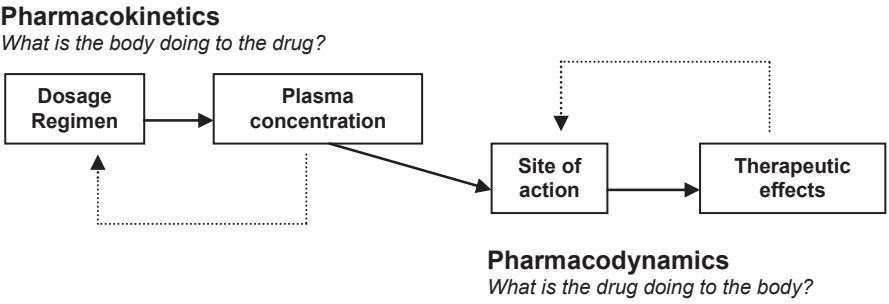

Fig. 1 The relationship between PK and PD in determining dosage regimens. The plasma–drug concentration data or effects produced are used via PK as a feedback (*dashed lines*) to modify dosage regimen to achieve optimal therapy

ficacy models in anticancer drug development area, to one where there is a truly scientific relation between preclinical and clinical pharmacology. The result thus expected would be one where there would be a reduction in the number of drugs being withdrawn from the market, due to unexpected and unacceptable adverse effects not picked up during drug development (cf. COX-II inhibitor class suits) [5].

At the same time, there was a parallel exponential shift in the understanding and role of genetics in pharmacology and the emergence of pharmacogenetics with the availability of cheaper and more rapid genetic analytical tests. This led to a new understanding that PK/PD differences may be due, in specific instances, to genetic variance [6]. Pharmacogenetics (PGx) was originally defined as the use of biological markers (DNA, RNA or protein) to predict the efficacy of a drug and the likelihood of the occurrence of an adverse event in individual patients. New definitions have emerged in recent years, yet the essential principles remain the same.

1.1 Succinylcholine: The First PK/PGx Study?

This parallel emergence of PK/PGx could not be better exemplified than in the emergence of the understanding of the differing mode of action of the muscle relaxant succinylcholine in some patients. In the 1950s it was clinically observed that patients differed in their response to this drug. It became known that the duration of this drug's action is determined by its enzymatic hydrolysis. It was only at a later stage that the genetic basis was discovered i.e. subjects homozygous for a gene encoding an atypical form of the hydrolysing enzyme, have a prolonged drug-induced muscle paralysis [7].

Other drugs followed suit. In the 1960s, it was discovered that a defect in N-acetylation metabolism of the drugs isoniazid (used for the treatment of tuberculosis), the antihypertensive drug hydralazine and the anti-arrythmic drug procainamide, could lead to prolonged half-life of these drugs in some patients, and this too was genetically determined [8].

The discovery in the mid-1970s, of the key role by the family of cytochromes, P450 system in drug metabolism, especially in hydroxylation, led, at the end of the 1980s, to an understanding of the molecular basis for slow metabolism in some patients, especially for those drugs which have a narrow therapeutic window [9].

Yet these developments remained only interesting academically. One of the key turning point in regulatory terms, however, was the discovery that irinotecan, which is used to treat bowel cancer, is metabolised by UDP-glucuronosyltransferase (UGT1A1). The *UGT1A1* promoter has a wild and variant copy (denoted *UGT1A1**28). This *UGT1A1**28 polymorphism is characterised by the presence of an additional TA repeat in the TATA sequence of the *UGT1A1* promoter, ((TA)7TAA, instead of (TA)6TAA). The latter causes a reduced UGT1A1 expression which leads to irinotecan toxicity. Today *UGT1A1* genotyping is mandatory in determining irinotecan dosage according to the FDA label for irinotecan, however in the EU the limitations of data available added to the reluctance to add a warning in several EU Member

States, i.e., it was not known what dose is the optimal one, in the *UGT1A1* WT as well as the *UGT1A1**28 [10].

More recently also for drug transporters the first genetic polymorphism was described which resulted in clinically relevant effects on the pharmacokinetics and adverse effects of some drugs, mainly statins, i.e., the *SLCO1B1* gene, encoding the OATP1B1 transporter protein [11].

1.2 Why Bother with PK/PGx?

Pharmacokinetic processes, like any aspect of human physiology, are inherently variable, and subject to both environmental and host-determined influences such as physiological and genetic factors. Genetic polymorphism (the occurrence in the same population of multiple allelic states) is responsible for a major proportion of the observed interindividual variability. These thus play a major role in PK/PD and therefore in dose response. Pharmacokinetic variation due to genetic factors can have the following consequences in any of the ADME processes such as:

- altered absorption/clearance
- difference in formation of active metabolites
- changes in drug interactions
- ethnic variation in drug response [12].

It is thus important to rationalise pharmacokinetic variation as exhibited by differing plasma concentration time curves in individual patients, on the basis of PGx effects and the interpretation of allelic changes. These need to be put into the context of PGx effect upon function e.g. one needs to be aware of how much an individual enzyme like CYP2D6 which is responsible for the metabolism of many drugs, contributes to the overall drug clearance. It is also important to consider what is the active moiety, the pathways affected and whether competing pathways could be present. In fact, PK/PGx studies would be key for those drugs which have a high inter-individual variation in efficacy and/or potency and have a narrow therapeutic range [12].

Thus, the ultimate goal of PK/PGx is to identify the contribution of genetic variability to differences in PK/PD, which in turn lead to individual differences in drug responses. This would enable prescribers to utilise a patient's PGx profile in order to select the drug which would exhibit the greatest efficacy and the least adverse effects in that specific patient and/or prescribe the drug at a dose which is appropriate for that patient i.e. personalised medicine [13].

In this review, a systematic discussion will be given on the PGx effect on the various PK processes i.e. ADME (absorption, metabolism, distribution and elimination). It will be shown how the development of a better understanding of PK/PGx guided principles, provide a crucial basis for the development of personalised medicines in individuals or specific subpopulations, optimising risk/benefit relationships, by maximising therapeutic efficacy with minimal adverse effects. It should

be kept in mind, however, that the fulcrum of PK/PGx relationships would be the clinical availability of reliable PGx test, and a strong relationship between genotype and phenotype. To date not all studies provide confirmatory evidence in this regard.

2 PK/PGx Concepts in the Absorption of Drugs

Absorption is no longer considered to be a passive mechanism and is now known to be the summation of extremely complex processes. Several membrane bound drug transporters, such as P-glycoprotein (P-gp, MDR1) and multidrug resistance (MDR) transporters, encoded by the *ABC* genes, have been identified as being responsible for the transport of drugs across membranes, especially those in the gastrointestinal tract following oral administration [14, 15].

These mechanisms have an important bearing on a drug's systemic bioavailability (F) which is used as a measure of how much drug eventually reaches the circulation after oral and any other non IV administration. Since the bioavailability of an intravenous drug dose is assumed to be 100 %, F is best calculated as the ratio of drug concentrations after giving the drug by the route of interest (usually oral) compared with the same dose given intravenously [15].

Sequencing of the *ABCB1* gene (which encodes P-gp) has shown that there are more than 50 single nucleotide polymorphisms (SNPs) for this gene, which vary in frequency according to ethnicity [16]. Wild-type *ABCB1* alleles have been associated with increased tissue expression of P-gp, and it has been suggested that the haplotype of three specific SNPs (1236C > T in exon 12; 3435C > T in exon 26 and SNP 2677G > (T, A) in exon 21) are more predictive of phenotype (i.e., reduced transport activity) than the individual SNP genotype.

A 3435C > T mutation rs1045642 linked to one of the other mutations has been found to result in a changed protein folding, which can change substrate binding[17]. However, the robustness of *ABCB1* genotype/phenotype association, has not yet been established despite many studies e.g. no direct influence has been found of the effect of MDR1 C3435T polymorphism on digoxin pharmacokinetics [18]. This greatly limits, to date, the use of *ABCB1* in PK/PGx.

3 PK/PGx Concepts in Distribution

Following administration, a drug is distributed into all of the body compartments and tissues that it is able to enter taking into account physical-chemical properties. The drug is said to distribute into an imaginary volume, called its volume of distribution, or Vd. This volume is imaginary because it is based on sampling drug concentrations in some reference fluid (usually serum or plasma) immediately after dosing, with the assumption that the entire dose of drug is uniformly distributed throughout the body. For drugs which partition into lipids, e.g. general anaesthetics,

plasma concentrations immediately after dosing will be quite low and the volume of distribution may appear to be many times larger than the volume of an average human being. Vd is essential for understanding where the drug goes and for estimating key parameters such as dose [12].

Vd is normally understood to be dependent on physiological parameters such as body mass index and fat deposits which may not have immediate PGx relations. However Vd may be PGx dependent in that distribution to certain body compartments, such as the brain across the blood brain barrier (BBB) and breast milk, may be dependent on transporters dependent on *ABC* genes, as outlined above. Overexpression of these *ABC* genes in certain patients may lead to drug efflux and what is clinically described as drug resistance [12].

Some studies suggest that the *ABCB1* variant 3435C>T rs1045642 affects plasma drug levels and drug resistance for drugs such as phenytoin, by-inhibiting transport [19]. In a study of British persons with epilepsy, the rs1045642 CC genotype was associated with drug resistance. In addition, a study of Egyptian persons with epilepsy showed increased likelihood of resistance to phenytoin in C allele carriers [20]. However, a meta-analysis failed to replicate the association with rs1045642, although many of these studies comprised patients on a variety of AEDs rather than phenytoin alone [21].

Also for the *SLCO1B1* gene, encoding OATP1B1, polymorphisms were demonstrated to result in clinically relevant effects on the pharmacokinetics, and more specifically the distribution, and adverse effects of some drugs, mainly statins. The *SLCO1B1**15 variant, 521T>C (Val174Ala) rs4149056 significantly affects the pharmacokinetics and adverse effects of many statins, and in a GWAS study indeed appeared to be associated with simvastatin-induced myopathy in patients treated for hypercholesteraemia. The prevalence of the *SLCO1B1**15 allele in the Caucasian population is 18% [11, 22].

Another key pharmacokinetic parameter in PK distribution is related to protein binding. Most drugs bind to plasma proteins to some extent and it may play a significant role in pharmacokinetics if it exceeds 80% (e.g. warfarin or phenytoin) as it is only the free (non-bound) drug which can exert a therapeutic effect. However to date while protein binding is an important pharmacokinetic parameter, at present no examples exists which point at PG affecting protein binding [15].

4 PK/PGx Concepts in Metabolism

There are over 170 genes known or expected to have a role in drug disposition, with more than half known to be polymorphic [23]. In pharmacokinetics, the highest level of polymorphism is found in genes involved in drug metabolism, especially cytochrome (CYP) P450 enzymes. These, in fact, account for over 80% of current PGx drug labelling requirements.

Several CYPs have been shown to be polymorphic as a consequence of single nucleotide polymorphisms (SNPs), gene deletions and gene duplications. Perhaps

the most studied is CYP2D6, which is involved in metabolism of approximately 100 drugs. More than 80 variants of CYP2D6 have been identified (http://www.cypalleles.ki.se), resulting in CYP enzymes with varying activities [24].

Examples abound in the literature of studies carried out on CYP2D6. For example it is responsible for the metabolism of codeine to the active metabolite, morphine. Thus the pharmacological activity of codeine is regulated by *CYP2D6* polymorphisms. In fact codeine has little therapeutic effect in patients who are CYP2D6 poor metabolisers, whereas due to excessive prodrug activation, CYP2D6 ultrarapid metabolisers suffer from adverse events due to increased levels of active metabolites. Thus *CYP2D6* genotype test results can be used to guide the dosing of codeine [25], and recently, information regarding the consequences of *CYP2D6* polymorphism have been included in the labelling of codeine.

However, even within the same drug class, genotype does not always predict drug metabolism. As an example, CYP2C19 is important in the metabolism of drugs such as protein pump inhibitors and an apparent gene–dose effect has been shown for the *CYP2C19**17 allele for pantoprazole, which predicts the plasma elimination rate constant, but this was not found for omeprazole. This difference could be to the difference in the contributions of CYP2C19 and CYP3A4 in the respective metabolism of the two drugs [26]. In fact, dependence on CYP2C19 metabolism is now seen by some as an undesirable property for NCEs in developments [6].

Polymorphisms in *CYP2D6* and *CYP2C19* have also been found to impact the metabolism of tricyclic antidepressents, such as amitriptyline and imipramine. These are demethylated by CYP2C19 to pharmacologically active metabolites, but then undergo further hydroxylation by CYP2D6 to less active metabolites. Thus polymorphisms in *CYP2D6* and *CYP2C19* may change the drug clearance or the ratio of parent drug to metabolites and dose adjustments can be estimated from the metaboliser status [27].

In oncology, the use of tamoxifen has for long been a mainstay in the adjuvant treatment of oestrogen receptor-positive breast cancer. Activity of tamoxifen is generally acknowledged to be mediated by the active metabolite endoxifen, which formation is catalysed by CYP2D6 [28–30]. Though the formation of endoxifen in CYP2D6 poor metaboliser patients is shown to be reduced, the consequences of the polymorphic status of *CYP2D6* for the success rate of tamoxifen treatment in relation to breast cancer recurrence or survival is not settled yet. In most cases these important clinical parameters have been investigated in fairly small studies, with only a small proportion of the known *CYP2D6* polymorphisms taken into account, whereas in some cases tumoral *CYP2D6* variations were assessed instead of germline variations, leading to a lack of Hardy–Weinberg equilibrium [31]. Though the totality of data are suggestive for a relationship between breast cancer recurrence and *CYP2D6* polymorphic status, more confirmative studies are needed, in particular with respect to the relationship between *CYP2D6* polymorphism and survival.

For clopidogrel, being a prodrug needing activation to an active metabolite mediated by CYP2C19, the efficacy may vary depending on the presence of specific functional allelic variants in patients. The conversion of the clopidogrel prodrug to active drug is strongly reduced in about 20 % of Asian patients being CYP2C19

poor metaboliser. This reduced metabolism results in less anti-coagulation and less protection against cardiovascular events [32, 33].

On the other hand, *CYP2C9* has two common variant alleles (*2 and *3); which, unlike CYP2D6 and CYP2C19, retain enzymic activity albeit at a reduced rate [19]. Thus *CYP2C9* polymorphisms only have a minimal impact on pharmacokinetics and thus generally no significant effect on therapeutic outcome. However, in the case of a drug with a narrow therapeutic index, such as warfarin, *CYP2C9* genotype has been shown to correlate with the titrated dose in a population of 200 patients [34]. It was found that the highest titrated dose, was in patients homozygous for the wild-type *1 allele, which has the highest activity, whilst the lowest titrated dose was in patients with *3 homozygotes, which have the lowest enzyme activity.

There may also be key ethnic differences in these CYP variants. For phenytoin, *CYP2C9**3 (rs1057910 A>C) is associated with decreased metabolism of this drug. However, the *CYP2C9**2 variant was found to be associated with decreased metabolism in patients with epilepsy, but not with phenytoin dose in a study of white persons with epilepsy. There are different *CYP2C9* variants (*CYP2C9**5, *6, *8 and *11) in black populations, which are linked with a decreased phenytoin metabolism. In Asian Indians, increased free phenytoin was found in *CYP2C9**3 carriers which led to an increased risk for concentration-dependent toxicity compared with *1 homozygotes [19].

Furthermore, in white populations, the frequency of carriers of the wild-type *CYP3A5**1 allele (showing CYP3A5 activity) is only about 15 %, whereas it is up to 50 and 90 % in Asians and Blacks, respectively [35]. In a meta-analysis [36] a clear effect of CYP3A5 on rejection rates was indeed concluded after the first month of the treatment with the immunosuppressant tacrolimus.

Hundreds of studies were carried out in this area in recent years. This has led to pharmaceutical companies screening out compounds, in drug development, to assess whether they are substrates solely for a known polymorphic enzyme in order to avoid the wider intersubject variability in exposure [26]. However, by doing this, it may be argued that one may end up relatively often with drugs in development which may be substrates for less studied genetic polymorphisms. Therefore, the EMA advocates that the involvement of known polymorphic enzymes and transporters should not prohibit further development of the drug, but instead should be taken into account during this clinical development, in order to provide satisfactory efficacy and safety in genetic subpopulations that have variable systemic exposure of active [37].

5 PK/PGx Concepts in Elimination and Clearance

Immediately after a dose of drug is administered, the body begins to eliminate or clear it. Most drug elimination follows first-order kinetics. That is, a constant fraction of drug is eliminated from the body during each unit of time and it assumes the

drug is uniformly distributed in a single body compartment with most of the drug eliminated from the body after four or five half-lives [12].

Clearance describes the rate at which the drug is eliminated from its volume of distribution, and its units are volume/time. Another important noncompartmental PK term is AUC, the area under the concentration-time curve. This term can be used to calculate overall clearance and half-life values for a drug. In addition, AUC is frequently used to compare drug exposures achieved with different drug doses, or to compare pharmacokinetics in the presence or absence of a drug with the potential to produce a PK drug interaction. It is to be pointed that drug systematic clearance is a summation of all the various organ clearances such as hepatic clearance renal clearance, salivary clearance, biliary clearance.

Renal clearance can be influenced by PGx differences especially for drugs which are eliminated mostly unchanged in urine. One such example is memantine, a frequently prescribed anti-dementia drug, which is mainly eliminated unchanged by the kidneys, partly via tubular secretion. Considerable inter-individual variability in plasma concentrations has been reported. A population pharmacokinetic study was performed in 108 patients who were genotyped for common polymorphisms in renal cation transporters (SLC22A1/2/5, SLC47A1, ABCB1). A SNP in NR1I2 (encoding the pregnane X receptor PXR) rs1523130 was identified as the unique significant genetic covariate for memantine clearance ($p = 0.006$), with carriers of the NR1I2 rs1523130 CT/TT genotypes presenting a 16% slower memantine elimination than carriers of the CC genotype [38].

6 PK and PGx in dose prediction

As has been described in the previous sections, several genetic polymorphisms have been identified in drug targets, drug-metabolising enzymes and drug transporters. Thus individual patients could theoretically be screened for specific polymorphisms, effectively acting as biomarkers, facilitating more specific and individualised choice of drug and dose (see Fig. 2). This strategy may enable therapeutic concentrations to be attained more quickly. However, it should be kept in mind that for a PGx test to be useful, the genotype must have a major influence on the PK/PD of a drug with a narrow therapeutic index.

As discussed in previous sections, genetic polymorphisms can result in changes to functional activity and PK through changes in expression of enzymes such as CYP3A5, UGT1A3, UGT2B17 and CYP2D6. This may result in important changes in clinical outcome which need to be noted.

Such genetic tests may be particularly useful in certain patients, such as renal transplant patients where PK/PD are hard to predict. PGx can be used to reduce the wide interindividual variation in the dose of immunosuppressive drugs required to achieve target blood concentrations, since PGx can be used to predict metabolism of these drugs, improving graft outcome. Several clinically useful strategies have

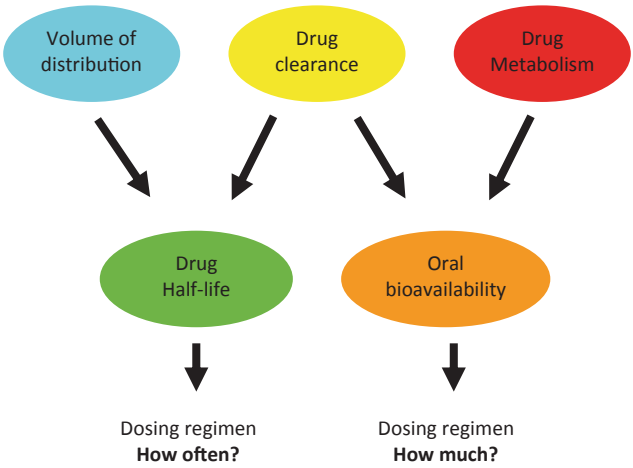

Fig. 2 The key pharmacokinetic parameters and their importance for designing dose regimen and dose size

emerged such as the use of the cytochrome P450 (CYP)3A5 (*CYP3A5*) genotype to predict the optimal initial dose for the immunosuppressant, tacrolimus [36, 39].

Warfarin has a narrow therapeutic index and a high dose variability. Thirty percent of this variance can be explained by SNPs in the warfarin drug target *VKORC1* and 12% by two non-synonymous SNPs (*2, *3) in CYP2C9. Affected individuals require, on average, lower doses of warfarin to maintain a therapeutic INR and more time to achieve stable dosing. A PK/PD model for warfarin, with *CYP2C9* and *VKORC1* genotype, age and target international normalised ratio (INR) as dose predictors has been developed [40]. Such a dosing algorithm may yield a more rapid dosing at the appropriate level, which is expected to reduce mortality of warfarin treatment. The actual effect of genotype-based dosing of warfarin during the initiation of therapy in patients with atrial fibrillation or venous thromboembolism has recently been tested in prospective clinical trials. In these cases warfarin was either prescribed according to a *CYP2C9*, *VKORC1* based dosing algorithm or the standard dosing regimen. The percentage of time that patients were in the therapeutic range for the international normalised ratio (INR) during the first weeks after warfarin initiation was measured. Results of these prospective studies however were not consistent, with some studies showing that genotype guided dosing was associated with a higher percentage of time in the therapeutic INR range than was standard dosing, whereas other reported that genotype-guided dosing of warfarin did not improve anticoagulation control during the first weeks of therapy during [41, 42]. Currently, EU drug regulatory agencies do not require genotyping before initiation of warfarin therapy, however, the warfarin drug label in the USA (Coumadin, FDA) [43] presents dosing information on the combined *VKORC1* and *CYP2C9* status that should be considered if this genotype is known prior to treatment.

7 What is the Role of PK/PGx Relationship in the Development of New Drugs?

As a result of the large output of high through put screening (HTS) in the evaluation of a new chemical entities (NCE), PK/PD relationships are established early on the drug development. PK/PD data are also used in the evaluation of preclinical studies and in the prediction of these parameters in actual patients. This optimises drug screening and reduces the risk of late stage attrition due to poor pharmacokinetics (see Fig. 3).

In fact, *in vitro* screening of a broad panel of *in vitro* metabolic or transport pathway evaluations for NCEs generally is determined early in preclinical evaluations. Such PK/PGx evaluations may trigger subsequent clinical PG-related investigations, e.g. by the inclusion of various PG variant patients in the clinical studies, in order to obtain an appropriate dose advice for the different important phenotypes for a certain polymorphic enzyme shown to be important in the pharmacokinetics of the drug. The PK/PGx evaluations are also important in the evaluation of drug interactions and dosing paradigms for desirable agents [4].

Recently the characterisation and development of pharmacophore template model for many CYPs such as for the active site of CYP2D6 has taken large steps forward. For example, this model has in fact been used in drug development of a novel

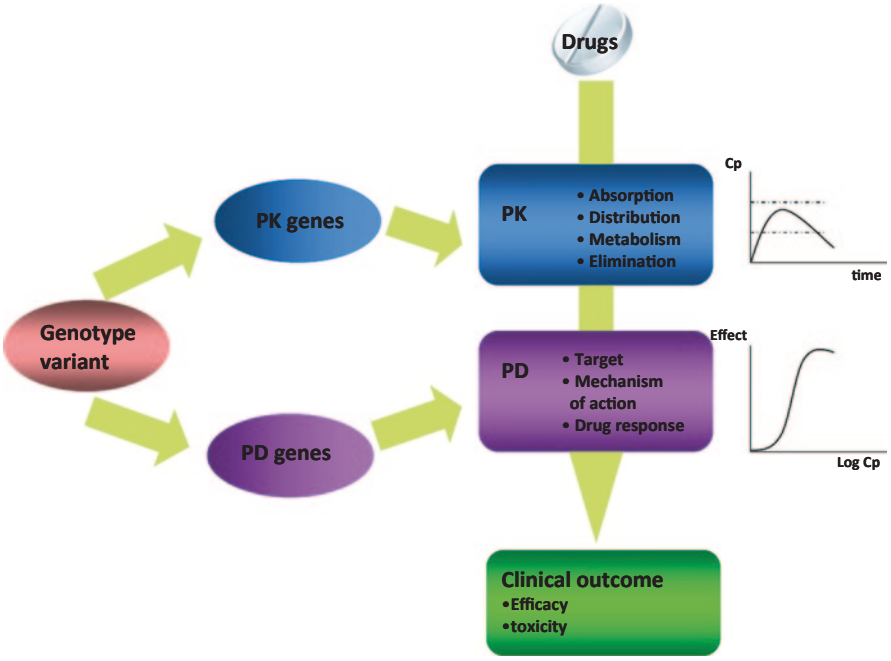

Fig. 3 Correlation of PK/PD and PGx in clinical outcome. By including PGx regarding PK and PD genes, PK and PD, and thus efficacy and safety of the drug may be optimised

calcium channel antagonist, together with in vitro data. These models indicated that the metabolism of this NCE was dependent on CYP2D6, and thus volunteers in the first in human study were genotyped for *CYP2D6*. Two volunteers were identified as poor metabolisers for CYP2D6 and were included in the study. The resulting PK data showed that the half-life of the drug was much higher in these individuals and it was decided to stop further development of the compound [6]. The current situation is that this is not considered a desirable approach anymore. Instead, a drug should be developed taking into account the various subpopulations. Indeed, stopping development because CYP2D6 is involved may seem wise on the short term, but on the longer term it may lead to the discovery that other, as yet unidentified polymorphic enzymes and transporters are important, which may appear only later in drug development, and was not anticipated. In that case, one is better off with a very well known polymorphic enzyme, to take into account just from the beginning of the development programme.

Go/no go decisions using such information from polymorphic enzymes, involves several approaches, many of them involving complex databases, drug–disease–trial models and simulation for the integration of information. Physiology-based pharmacokinetic (PBPK) models have also been developed which provide a very useful mechanistic approach to drug development. This plethora of information may result in a number of conflicting information which may determine issues such as when it may be best to stop the development especially if there are back ups without the potential issue? While data can be generated on different doses, this greatly confounds the drug development program. In addition, the decision to terminate often considers multiple aspects such as the known frequency of polymorphism, the fraction metabolised through pathway, regulatory and commercial pressures, therapeutic window of the drug, the indication and unmet medical needs, alternative current treatment options.

Moreover even if 2D6 is identified, other polymorphic pathways may play key roles in the development of that compound. It may be valid for some time that it would be best to develop a drug with a well known polymorphism but this can only be done in conjunction with all the other factors as discussed in previous sections.

8 PK/PGx Concepts in Drug Regulatory Guidelines

Drug regulatory authorities, such as the Food and Drug Administration (FDA) through the *Interdisciplinary Pharmacogenomics Review Group IPRG* and European Medicines Agency (EMA) through the *Pharmacogenomics Working Party PGWP* have for some time been establishing guidelines for submission of pharmacogenetic data on therapeutic drugs to assist in the tailoring of drug therapy to individual patients.

These agencies now request PK/PGx information in the labelling for several drugs. For example, in 2007, FDA (but to date not EMA) issued a labelling change advising physicians to consider the use of “genetic tests to improve their initial

estimate" of the dose of anticoagulant drug warfarin, which is widely prescribed for reducing the risk of thrombosis and its complications [44]. This recommendation has set a precedent for the use of genetic technologies in clinical practice and now several bodies are pushing for such novel technology to enhance personalised medicine. Likewise, recently, information regarding the consequences of *CYP2D6* polymorphism have been included in the labelling of codeine. Presently, there are over 70 licensed drugs with PGx labels, where the polymorphic *CYP2C9, CYP2C19* and *CYP2D6* account for the majority of these labels. In fact IPRG and PGWP now liaise together with industry for combined VGDS—Voluntary Genomic Data Submissions/Pharmacogenomic briefing meetings in order to streamline regulatory requests in this fast developing area.

Further, the EMA PGWP published a guideline on the role of pharmacogenetics in PK in 2012 [37] soon followed by a guidance on this topic by the FDA (Guidance on Clinical Pharmacogenomics: Premarketing Evaluation in Early Phase Clinical Studies) [44]. These guidelines request the drug developers to identify those pharmacogenomic factors that may affect safety and/or efficacy of drugs that are currently being developed. For that purpose, the consequences of pharmacogenomic variation should be investigated if *in vitro* and/or clinical (*in vivo*) studies indicate that a known functionally polymorphic enzyme or drug transporter is likely to be important in the disposition of the drug, or if these represent an important factor in the formation, elimination or distribution of a pharmacologically active or toxic metabolite. Pharmacogenomic investigations are also required when clinical studies indicate that major interindividual differences in the pharmacokinetic properties (that cannot be explained by other intrinsic or extrinsic factors) are likely to influence the efficacy or safety of the drug in a genetically variable subpopulation. When looking at a global level, there appears broad agreement on the requirements with respect to pharmacogenomics related to pharmacokinetics in the EU, USA and Japan, though some divergence still exists on some areas, like the actual cut-off which would trigger the need for *in vivo* pharmacogenomic investigations, and the stringency by which banking of DNA samples from ongoing clinical studies is required [45]. Overall, however, it is clear that in the future, for new drugs, more pharmacogenomic data is expected to become available which will enable appropriate dosing in e.g. patients with a different metaboliser status, than has been in the past.

9 Outlook and Recommendations

The better understanding of pharmacogenetics on PK/PD inter-individual variability of drug disposition might be beneficial in the context of individual dose optimisation in personalised medicine. The greatest understanding has been in that of metabolic phenotyping especially of metabolising enzymes. The application of PGx to predict other PK processes and thus dosage regimens depends, however, on various other cofounding factors such as disease and co-administered drugs which limits the feasibility of clinical applications to date. This will determine how much

a genetic variant contributes to a clinically significant pharmacokinetic variability overall.

There is now a growing recognition that the future of the pharmaceutical industry will depend a great deal on the integration of PGx and PK/PD data to guide drug decision-making. Novel PGx biomarkers are important to fill in gaps of uncertainty about therapeutic targets, variability in drug response; algorithm-based dose determination; response monitoring; early indicator/predictor of toxicity/adverse reactions. The debate still remains on the adoption of PGx assays in clinical examinations and the implications of reimbursement. Recent data on warfarin and clopidogrel have identified barriers to successful implementation [46]. The data available for such technology varies a great deal but used with agreed clinical guidelines, appears to be the strongest predictor of reimbursement. However bringing better clinical evidence is needed.

It important to note, however, that other factors can also influence PK/PD processes which may impact the predictability of outcomes in these patients, such as co morbid medical conditions, smoking, diet, drug interactions, race and frailty.

Integrating PK/PD with PGx can be our magic ball in the determination of drug doses, dosing intervals, titration regimens in order to decrease the risk of drug adverse events and toxicity and ensure successful outcomes in patients. Further knowledge is likely to add to our understanding of differences in sub-populations, but the potential limitations of these approaches should be recognised in order that they can be applied beneficially [6].

The availability of open access on-line resources such as PharmGKB, the pharmacogenomics database, and simulation models such as SIMCYP® have greatly facilitated the availability of resources which systematically assess the vast information now available on the impact of genetic variation on drug response for clinicians and researchers [47].

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