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Addressing phenoconversion: the Achilles' heel of personalized medicine

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Phenoconversion is a phenomenon that converts genotypic extensive metabolizers (EMs) into phenotypic poor metabolizers (PMs) of drugs, thereby modifying their clinical response to that of genotypic PMs. Phenoconversion, usually resulting from nongenetic extrinsic factors, has a significant impact on the analysis and interpretation of genotype-focused clinical outcome association studies and personalizing therapy in routine clinical practice. The high phenotypic variability or genotype–phenotype mismatch, frequently observed due to phenoconversion within the genotypic EM population, means that the real number of phenotypic PM subjects may be greater than predicted from their genotype alone, because many genotypic EMs would be phenotypically PMs. If the phenoconverted population with genotype–phenotype mismatch, most extensively studied for *CYP2D6*, is as large as the evidence suggests, there is a real risk that genotype-focused association studies, typically correlating only the genotype with clinical outcomes, may miss clinically strong pharmacogenetic associations, thus compromising any potential for advancing the prospects of personalized medicine. This review focuses primarily on co-medication-induced phenoconversion and discusses potential approaches to rectify some of the current shortcomings. It advocates routine phenotyping of subjects in genotype-focused association studies and proposes a new nomenclature to categorize study populations. Even with strong and reliable data associating patients' genotypes with clinical outcome(s), there are problems clinically in applying this knowledge into routine pharmacotherapy because of potential genotype–phenotype mismatch. Drug-induced phenoconversion during routine clinical practice remains a major public health issue. Therefore, the principal challenges facing personalized medicine, which need to be addressed, include identification of the following factors: (i) drugs that are susceptible to phenoconversion; (ii) co-medications that can cause phenoconversion; and (iii) dosage amendments that need to be applied during and following phenoconversion.

Pharmacogenetics and personalized medicine

The majority of drug-metabolizing enzymes (DMEs) are subject to genetic polymorphism and show some degree of functionally significant polymorphism in the population. The clearest data in this regard relate to cytochromes P450 CYP2D6 and CYP2C19 and thiopurine methyltransferase (TPMT). These genetically determined polymorphisms give rise to three distinct genotype-based subpopulations with respect to each DME, namely extensive metabolizers (EMs), poor metabolizers (PMs) and a subgroup in between, the intermediate metabolizers (IMs). In addition, for CYP2D6 and CYP2C19, there is a fourth genotype, the ultrarapid metabolizer (UM) genotype, resulting from inheritance of either multiple copies of the functional wild-type allele, such as *CYP2D6*1*, or a higher metabolic capacity resulting from increased transcription of DME due to the presence of a genetic variant found in the promoter region of *CYP2C19* that defines the

*CYP2C19*17* allele [1, 2]. The resulting intergenotype variability in pharmacokinetics of certain substrates and their metabolites [3] is often believed to account for a substantial fraction of intergenotype variability in drug response (efficacy and/or safety). The frequency of variant alleles that give rise to these genotypes, estimated by population-based studies in healthy volunteers following administration of a DME-specific probe drug, is known to display major interethnic differences [4, 5].

This potential for intergenotype differences in drug response has also stimulated large-scale population-based pharmacogenetic association studies in patients, both retrospective and prospective, in order to establish possible associations between commonly prevalent DME genotypes and clinical outcomes (clinical phenotype) following pharmacological therapeutic interventions. These studies have almost always focused on the DME genotypes of the study population with the assumption that genotypes of all the study subjects predict their functional phenotype, and further that despite wide inter- and intra-genotype variability in metabolic capacity, clinical responses are simple binary outcomes associated with discrete genotype groups. The aim of these studies is to examine the strength of these associations between genotype and clinical phenotype and, if shown to be strong, to develop a dosing regimen appropriate to each genotype [6]. This concept is the so-called '*personalized medicine*', which is a widely promoted under the slogan of 'the right drug at the right dose the first time'. Personalized medicine (better and more accurately promoted as 'personalized dose') is expected to displace the traditional 'one-size-fits-all' paradigm to pharmacological interventions, thereby making therapy not only more effective but also much safer.

Recent high-profile association studies

The last decade has witnessed much interest and effort in evaluating possible associations between patients' DME genotypes and clinical outcomes (clinical phenotype) following the use of a number of high-profile drugs. Drugs with a narrow therapeutic index and/or those intended for the treatment of life-threatening diseases lend themselves well to such studies. Such drugs include warfarin (*CYP2C9* genotype and risk of haemorrhage or stroke), tamoxifen (*CYP2D6* genotype and risk of therapeutic failure), clopidogrel (*CYP2C19* genotype and risk of thrombotic cardiovascular outcomes), irinotecan (*UGT1A1* genotype and risk of diarrhoea or myelosuppression) and thiopurines (*TPMT* genotype and/or phenotype and risk of myelosuppression). It is noteworthy that while TPMT association studies have tested TPMT status by genotyping and/or phenotyping for the enzyme activity in red blood cells prior to initiating therapy with thiopurines, the vast majority of the P450-based DME association studies have focused exclusively on patients' DME genotype.

This emphasis on correlating the DME genotype of patients with clinical outcomes carries a risk that requires attention, namely the consequences of co-medication-induced phenoconversion, whereby a genotypic EM has the DME phenotype of a PM. This phenomenon is usually recognized clinically as drug–drug interactions that are common in patients, some with serious clinical consequences, but as discussed below, comorbidity may also give rise to genotype–phenotype mismatch. In view of the differences in prevalence of the use of interacting co-medications and presence of comorbidity [such as human immunodeficiency virus (HIV) infection, liver disease or cancer], we emphasize the fundamental distinction between these patient-based association studies from those in healthy volunteers, which are aimed at estimating the genotypic structure of a population by inferring the genotype from the metabolic phenotype of probe drugs.

Despite strong mechanism-based pharmacological support, the results from genotype-based association studies have been inconsistent and often conflicting, and it is therefore timely to explore phenoconversion as one the potential explanations for this.

Pitfalls and challenges associated with emphasis on genotype

Genotyping and its interpretation have proved to be far more complex and challenging than previously believed. As of 2 March 2014, the numbers of alleles described were no less than 105 for CYP2D6 (29 with no or negligible activity), 58 for CYP2C9 (four with no activity) and 34 for CYP2C19 (six with no activity) [http://www.cypalleles.ki.se/]. In addition, there are a number of additional single nucleotide polymorphisms for each, where the haplotypes have not yet been determined. In a majority of association studies, only the genotype is considered as a determinant of outcome and only the commonly prevalent alleles are interrogated for when assigning a genotype. However, any commentary on the pitfalls of emphasizing and associating genotype with a clinical outcome must begin by reiterating the obvious that it is not the genotype *per se* but the DME phenotype it expresses that determines the clinical outcome following a pharmacological intervention.

Assumption of a DME phenotype from its genotype is proving to be increasingly challenging, considering the ever-growing number of alleles and the wide range of their functional expressions. Furthermore, there is no standardized process by which to translate a genotype into a phenotype assignment. This is very evident for *CYP2D6* genotype, and The Dutch Pharmacogenetics Working Group has issued a table for inferring the CYP2D6 phenotype from a combination of various *CYP2D6* diplotypes [7].

In an effort to streamline genotype interpretation, Gaedigk *et al*. [8] also introduced an activity score (AS) system in 2008, which has since gained acceptance among the scientific community and has also been adopted by the Clinical Pharmacogenetics Implementation Consortium. The AS system assigns, for each allele, a value of 0 for null, 0.5 for intermediate, 1.0 for wild-type and two times these scores for the corresponding gene duplication genotypes. The AS of a genotype is the sum of the values assigned to each allele. For example, *CYP2D6*1/*1* and *CYP2D6*2/*5* genotypes have AS of 2 (1 + 1) and 1 (0.5 + 0.5), respectively. The AS is then used to place an individual into a continuum of activity scores (rather than assigning the individual to a discrete and traditional UM, EM, IM and PM category), which (together with data on ethnicity) enables prediction of the probable phenotype of the individual. The AS system predicts that the majority of Caucasian subjects with null/functional genotypes (AS of 1) will be slow EMs (73% chance), but also emphasizes that a given subject with this score could be an IM (17%), a fast EM (9%) or even an UM (2%), although less frequently. The reader is referred to the excellent reviews of the complexities and challenges this poses for CYP2D6 genotyping [8–10]. More importantly, however, Gaedigk *et al*. [8] also acknowledge that factors that modulate DME activity, such as CYP2D6 inhibitors, would always need to be considered, regardless of whether the genotype or the AS system is used for predicting the phenotype.

With regard to *CYP2D6* genotype and metabolic activity of CYP2D6, a further challenge is presented by some data which suggest that the functional consequences of allelic variation in *CYP2D6* may be substrate dependent and thus may be reflected in pharmacokinetic consequences for individuals [11, 12]. Thus, although it is now possible to genotype subjects with a high level of accuracy and/or assign an AS, even the availability of high-quality genotypic data may mislead prescribers because there is poor understanding of the corresponding DME phenotype. Of course, a DME PM phenotype is inferred with confidence when the subject has only a diplotype encoding for no or hardly any metabolic activity. Furthermore, in respect of non-PM genotypes, there is also poor understanding of the extent to which different co-medications and other factors, such as comorbidity, can modulate the activity of a DME.

Co-medications as causes of phenoconversion

The metabolic activity of a DME, typically expressed as the metabolic ratio (of the concentrations of parent drug to metabolite) of the probe drug, is modulated not only by the heritable traits but also by drugs. Just as genetically inherited variant traits give rise to DMEs of altered activity, administration of certain drugs can also inhibit a DME, mimicking the genetic defect and producing a PM phenocopy [13, 14], or induce the DME and give rise to an EM or UM phenocopy [15].

This drug-induced mimicry of congenital defects (phenoconversion) is a well-known phenomenon in other areas of medicine. For example, both acquired and congenital prolongation of the QT interval of the electrocardiogram result from inhibition of the α-subunit of the delayed rectifier potassium channel; the former typically by drugs and the latter by mutations in the gene (*KCNH2*) encoding for this subunit [16]. For the patient, however, the clinical consequence of both forms is a potentially fatal proarrhythmia known as torsade de pointes [17]. Improved understanding of the defects underpinning some genetic diseases has also led to the development of novel therapeutic agents; for example, the therapeutic introduction of sodium–glucose cotransporter linked protein (SGLT2) inhibitors for the treatment of type 2 diabetes [18] and cholesteryl ester transfer protein (CETP) inhibitors for the treatment of dyslipidaemia [19], mimicking congenital forms of renal glycosuria and raised high-density lipoprotein cholesterol, respectively.

Likewise, administration of therapeutically used drugs can also give rise to acquired forms (phenocopy) of impaired drug metabolism normally associated with heritable traits. A wide variety of drugs from a whole range of pharmacotherapeutic classes are known to inhibit DMEs [20], thereby increasing the exposure to the parent substrates of these DMEs with a corresponding decrease in exposure to their metabolites (some of which are often pharmacologically active). Table 1 is a compilation, albeit not an exhaustive one, of some of the better known inhibitors and inducers (or phenoconverters) of various DMEs. For further details, the reader is referred to a review by Samer *et al*. [21]. Indeed, some over-the-counter medicines are also well-known CYP inhibitors (e.g. diphenhydramine, omeprazole and cimetidine). These over-the-counter medicines [22] and a few herbal remedies that also modulate CYP enzyme activity [23–26] are rarely taken into account in research studies or clinical settings. The resulting high phenotypic variability and the extent of genotype–phenotype mismatch in the genotypic EM group mean that many of these genotypic EMs would behave pharmacologically as PMs and therefore, in many clinical situations and association studies, the number of phenotypic PM subjects may be greater than predicted from genotype if only genotypic approaches are used to categorize subjects as EMs or PMs.

At the risk of stating the obvious, phenoconversion and its duration are typically related to the dose and duration of co-administration of the DME inhibitor. For example, in healthy *CYP2D6* EM volunteers, a daily 20 mg dose of fluoxetine increased the dextromethorphan metabolic

Table 1

Examples of some inhibitors and inducers of major polymorphic drug-metabolizing enzymes

DME	Inhibitors		Inducers
CYP2C9	Allopurinol Anastrozole Diclofenac Fluvoxamine Fluvastatin Metronidazole Sulfinpyrazone Zafirlukast	Amiodarone Cimetidine Fluconazole Flurbiprofen Isoniazid Trimethoprim Voriconazole	Rifampicin Bosentan Carbamazepine St John's wort Ethanol
CYP2C19	Chloramphenicol Felbamate Fluvoxamine Ketoconazole Modafinil Paroxetine Ritonavir Tolbutamide	Cimetidine Fluoxetine Indomethacin Lansoprazole Omeprazole Probenecid Ticlopidine Topiramate	Rifampicin
CYP2D6	Amiodarone Cinacalcet Duloxetine Fluoxetine Halofantrine Methadone Paroxetine Ritonavir Sertindole Thioridazine	Bupropion Diphenhydramine Flecainide Fluphenazine Haloperidol Moclobemide Quinidine Sertraline Terbinafine	None known
UGT1A1	Atazanavir Ketoconazole Nilotinib Pazopanib	Indinavir Sorafenib Erlotinib	Rifampicin Phenytoin Carbamazepine
TPMT	5-Aminosalicylic acid Thiazide diuretics	Frusemide Mesalamine Sulphasalazine NSAIDs	None known

Abbreviations are as follows: DME, drug-metabolizing enzymes; NSAIDs, nonsteroidal anti-inflammatory drugs; TPMT, thiopurine methyltransferase. [Compiled from literature review.]

ratio (DMR), a well-established measure of CYP2D6 activity, by a factor of 9.1 after 7 days and 17.1-fold after 28 days of treatment [27], whereas a 50 mg daily dose of cinacalcet for 8 days increased the exposure and peak concentrations of dextromethorphan to 11- and 7-fold, respectively [28]. In a study by Pope *et al*. [29], urinary DMR showed a quinidine dose- and time-related increase in the number of *CYP2D6* EM subjects converted to the PM phenotype that reached 100% on day 3 of dosing with 25 mg quinidine. Even genotypic UMs may be at risk of being converted into phenotypic normal EMs or PMs by paroxetine [30]. Dalén *et al*. [31] were able to establish a dose–effect relationship for quinidine-induced inhibition of CYP2D6 in UMs. Lam *et al*. [32] have also reported that for paroxetine and fluoxetine, plasma concentrations and dosage of these CYP2D6 inhibitors strongly influence the magnitude of enzyme inhibition.

Comorbidities as causes of phenoconversion

Apart from drugs, other factors can also result in DME phenoconversion. For example, in some HIV-positive patients, CYP2D6 activity approaches that of PMs, despite having an EM genotype [33]. Jones *et al*. [34] reported that compared with age- and sex-matched healthy volunteers, HIV-infected subjects had 90% lower CYP2D6 activity, 53% lower N-acetyltransferase-2 (NAT2) activity and 18% lower hepatic CYP3A4 activity. There was CYP2D6 genotype-phenotype discordance in 4 of the 5 phenotypic PM HIV-infected subjects.

As it concerns hepatic drug metabolism, liver transplant can also result in the apparent conversion of many PMs into EMs and on rare occasions, EMs into PMs [35, 36]; in these circumstances, determining a patient's genotype by analysing nonhepatic tissues will give erroneous information on the patient's metabolic capacity.

Liver disease is another important cause of phenoconversion [37, 38]. Among 30 patients without concomitant drug intake, only one subject was identified as *CYP2C19* PM (3.3%) using both mephenytoin and omeprazole as the metabolic probes. However, 30 (64%) of 47 patients with liver disease and 20 (18%) of 110 co-medicated patients without liver disease had PM phenotype, highly exceeding the PM frequency of 3–4% in Caucasians [37].

Williams *et al*. [39] have reported discordance between *CYP2C19* genotype and phenotype (expressed enzyme activity) in cancer patients without any known cause for this discrepancy. Among 16 cancer patients with *CYP2C19* EM genotype were four (25%) with PM phenotype and in the other 12, there was a general shift of omeprazole metabolic ratio towards a slower metabolic activity. More recently, using omeprazole as the probe drug for determining the phenotype, Helsby *et al*. [40] have also reported that the activity of CYP2C19 was severely compromised in advanced cancer patients, resulting in a PM status in 37% of the patients who had normal EM genotype, and emphasized that in a cancer population, genotyping for *CYP2C19* would significantly underestimate the number of phenotypic PMs of CYP2C19 substrates.

Indication-related potential for phenoconversion

An ironic aspect of drug-induced phenoconversion is that for some indications and drugs of great interest from the perspective of association studies and personalized medicine, a number of patients require concurrent medications that result in phenoconversion. For example, omeprazole (a CYP2C19 inhibitor) is commonly co-prescribed with clopidogrel (metabolized by CYP2C19) as gastrointestinal

ulcer prophylaxis against increased risk of bleeding. Likewise, paroxetine or fluoxetine (both potent CYP2D6 inhibitors) are often prescribed with CYP2D6-metabolized tamoxifen to treat tamoxifen-induced hot flushes. Recently, the US Food and Drug Administration have approved paroxetine for the treatment of moderate to severe hot flushes (vasomotor symptoms) associated with menopause. Selective serotonin reuptake inhibitors (SSRIs), such as paroxetine, may also be prescribed to treat depression which may occur as a comorbidity or be induced by tamoxifen in breast cancer patients. Likewise, amiodarone (a CYP2C9 inhibitor) is often co-administered to control atrial fibrillation in patients who are also candidates for warfarin therapy widely used in the same indication. Patients receiving warfarin may also be prescribed fluvastatin, another potent CYP2C9 inhibitor, to control coexisting hyperlipidaemia. As a result of such co-prescriptions, however appropriate clinically, association studies in such indications, where the use of such drug pairs is likely, have greater potential for failing to detect a clinically relevant association, if one genuinely exists.

Concern regarding drug-induced phenoconversion is illustrated by routine phenotyping for TPMT activity. The TPMT phenotype may be affected by a number of factors, such as a recent blood transfusion or co-medication with drugs such as sulfasalazine, mesalamine and olsalazine. Therefore, when patients are phenotyped for their TPMT status by measurement of erythrocytic enzyme activity, those with deficient TPMT status are subsequently genotyped to confirm their TPMT status. Although this may be explained partly by ready access to the phenotyping procedure and cost, a likely additional explanation is that these patients may commonly be receiving a number of drugs that are known to inhibit TPMT activity, and their co-prescription may change during thiopurine treatment with dire consequences for therapeutic failure or profound myelosuppression.

Pharmacokinetic and clinical consequences of phenoconversion

Table 2 is a summary of typical pharmacokinetic consequences that follow in a genotypic *CYP2D6* EM following drug-induced phenoconversion to a CYP2D6 phenotypic PM [41]. For more detailed data on the pharmacokinetic changes that could follow phenoconversion, the reader is referred to reviews by Michalets [42], Cavallari *et al*. [43] and Hirota *et al*. [44].

It is evident that phenoconversion may result in dramatic pharmacokinetic consequences for some drugs, typically increasing the exposure to the CYP2D6 substrate drug by a factor of two to three, with a corresponding decrease in the exposure to the metabolite(s), but these changes may be even more marked for some drugs [41].

Table 2

Examples of pharmacokinetic consequences of phenoconversion following drug-induced inhibition of CYP2D6

Phenoconverting inhibitor	Substrate	Ratio of substrate AUC with and without inhibitor
Quinidine	Mexiletine	1.32
	Imipramine	1.54
	Propafenone	2.70
	Propranolol	1.98–3.00
	Metoprolol	3.24
	Desipramine	7.50
	Encainide	11.40
	Dextromethorphan	13–49 (depending on the dose of quinidine)
Fluoxetine	Propafenone	1.50
	(*R*)-Carvedilol	1.77
	Imipramine	3.33
	Desipramine	4.80
	Tolterodine	4.84
Paroxetine	Tamsulosin	1.64
	Imipramine	1.74
	Aripiprazole	2.36
	(*R*,*S*)-Metoprolol	4.21–6.16
	(*S*)-Metoprolol	5.08
	Desipramine	4.64–5.21
	Atomoxetine	6.50
	Perphenazine	6.96
	(*R*)-Metoprolol	7.93
Terbinafine	Paroxetine	2.50
	Desipramine	4.94
Cinacalcet	Desipramine	3.6
	Dextromethorphan	11.5

Abbreviation is as follows: AUC, area under the plasma concentration *vs*. time curve. [Compiled from literature review.]

Such pharmacokinetic changes can adversely impact the safety and/or efficacy of the substrate drug [45], depending on the pharmacological activities and the therapeutic indices of the parent compound and its metabolites. For example, phenoconversion of a genotypic EM into a phenotypic PM by quinidine or fluoxetine markedly reduces the activation of pharmacologically inactive codeine into its pharmacologically active analgesic metabolite, morphine, thereby often resulting in therapeutic failure [46], whereas perphenazine toxicity is common in paroxetine-induced phenotypic PMs with EM genotype [47]. For the high-profile drugs listed earlier (in the section entitled 'Recent high-profile association studies'), the consequences for association studies arising from such DME genotype–phenotype mismatch hardly need to be spelled out. It is therefore not surprising that an extensive package of all relevant drug–drug interaction studies is now mandatory for all new drugs seeking regulatory approval. Neither is it surprising that regulatory guidance from the European Medicines Agency on the use of pharmacogenetic methodologies in the

Table 3

Reported rates of drug-induced phenoconversion of genotypic CYP2D6 extensive metabolizers to phenotypic CYP2D6 poor metabolizers

Co-medication	Probe drug for phenotyping	Total subjects studied	Phenoconversion rates (%)	References
Antipsychotics	Dextromethorphan	14	21	[138]
A wide range	Venlafaxine	865	24	[60]
Bupropion	Dextromethorphan	13	46	[139]
		1	100	[140]
Methadone	Dextromethorphan	28	57	[141]
Quinidine	Debrisoquine	7	100	[13]
	Debrisoquine	6	67	[14]
Terbinafine	Dextromethorphan	6	67	[142]
		10	100	[54]
Thioridazine	Debrisoquine	14	71	[59]
Paroxetine	Dextromethorphan	30	80	[143]
Paroxetine, moclobemide and metoprolol	Dextromethorphan	??	100	[144]

pharmacokinetic evaluation of medicinal products also emphasizes that in the context of drug response, genetic subpopulations include not only those characterized by genotype but also by their phenotype [48].

Potential scale of the phenoconversion problem

In terms of the potential scale of phenoconversion in the population at large, we focus primarily on CYP2D6 because it is the most widely studied DME in this respect.

It is believed that 20% of all drugs metabolized by cytochrome P450s are CYP2D6 substrates [49] and they include drugs with narrow therapeutic indices commonly used in clinical practice today, such as the cardiovascular and psychoactive drugs. Amongst the most potent CYP2D6 inhibitors are the SSRI antidepressants fluoxetine and paroxetine, both of which are widely used clinically. In terms of the scale of the use of SSRIs alone as an example, one study in Scotland reported that the percentage of the population receiving SSRIs had increased from 8.0% in 1995–1996 to 11.9% in 2000–2001, with a simultaneous increase in treatment duration and mean daily doses [50]. In terms of their co-medication with CYP2D6 substrates, Preskorn *et al*. [51] have reported that of the 461 patients receiving these SSRIs, 39 (8%) were also receiving a CYP2D6 substrate drug with a narrow therapeutic index. The scale of genotype–phenotype mismatch in the wider population and its implications for genetic association studies can best be illustrated by CYP2D6.

Frequency of phenoconversion

Although there are hardly any large-scale population studies on the potential scale of phenoconversion-mediated genotype–phenotype mismatch, evidence from a number of small clinical studies that have simultaneously examined the genotype and the phenotype of the study subjects suggests that the problem is most likely to be a significant one and that the phenomenon of drug-induced phenoconversion should be a matter of concern.

Among the first and prototype probes for determining CYP2D6 metabolic activity were debrisoquine and sparteine. The influence of co-medications on a DME phenotype, and its potential clinical consequences for genotype-based predictions, can best be judged from a study which reported that patients receiving SSRIs, antipsychotics or other drugs known to be the substrates or inhibitors of CYP2D6 showed a significantly higher mean sparteine metabolic ratio than their untreated counterparts [52]. Table 3 summarizes the reported rates of phenoconversion of *CYP2D6* EM genotype to CYP2D6 PM phenotype in subjects receiving a variety of CYP2D6 substrates and/or inhibitors and studied using a variety of phenotyping probe drugs.

Duration of phenoconversion

Although phenoconversion is transient for the duration of inhibition of the DME, the effect of some inhibitors, such as quinidine or fluoxetine, can persist for days after they are discontinued [14, 27, 53]. This is related to the half-life of the inhibitor and its affinity for the DME it inhibits. Table 4 summarizes the reported duration of CYP2D6 inhibition after the last dose of the phenoconverting co-medication.

The intensity of inhibition may also be sensitive to ethnicity, which is probably also a genetic effect [54–56].

Genotype-dependent susceptibility to phenoconversion

Mechanistic considerations dictate that individuals with IM genotype are likely to be more susceptible to phenoconversion due to their intrinsically already compromised capacity to mediate drug metabolism. In contrast, individuals with PM genotype are unlikely to be susceptible to phenoconversion. Regardless of

Table 4

Reported duration of drug-induced CYP2D6 inhibition after the last dose of the phenoconverting medication

Co-medication	Duration of phenoconversion after the last dose of inhibitor	References
Quinidine	At least 3 days	[14]
	21 days	[53]
Fluoxetine	>2 weeks	[27]
Fluoxetine	63.2 ± 5.6 days	[145]
Paroxetine	20.3 ± 6.4 days	
Sertraline	25.0 ± 11.0 days	
Terbinafine	>4 weeks	[146]
Paroxetine	4 weeks after 6 weeks of treatment	[147]
	6 weeks after 18 weeks of treatment	

co-prescription of interacting drugs, an individual with *CYP2D6*4/*4* (PM) genotype would not benefit from codeine, and an individual with a *TPMT*2/*2* genotype would be at the greatest risk of developing severe myelosuppression if prescribed normal doses of a thiopurine, because of already low or absent baseline metabolic capacity. However, exceptions may occur even in PMs in whom alternative pathways which are subject to inhibition or induction are active. This has been demonstrated elegantly by rifampicin-induced induction of a CYP3A4-mediated alternative pathway activated by *CYP2D6* PMs to eliminate propafenone [57].

In one small study, the increase in the metabolic ratio of phenformin, a CYP2D6 substrate, following CYP2D6 inhibition was the greatest in subjects whose baseline metabolic ratio was consistent with heterozygous or IM genotype [58]. In another study, by Llerena *et al*. [59], the inhibition of debrisoquine metabolism by thioridazine was genotype dependent. Patients with *CYP2D6* IM genotype were phenoconverted into PM phenotype at a lower dose (50 mg day^{-1} or greater) of thioridazine than those of *CYP2D6* EM genotype (150 mg day^{-1}). In the study by Preskorn *et al*. [60], phenoconversion to CYP2D6 PM phenotype occurred in 159 (21.3%) of 748 genotypic EM subjects and in only one (2.8%) of the 36 genotypic UM subjects. Lam *et al*. [32] have also reported that the potential of paroxetine as an inhibitor may be affected by the genotypes and basal metabolic capacities of individual subjects. With regard to CYP2C9, Kumar *et al*. [61] found that the apparent oral clearance of flurbiprofen differed significantly among the three *CYP2C9* genotype groups at baseline but not after pretreatment with 400 mg fluconazole for 7 days and concluded that the presence of *CYP2C9*3* alleles (either one or two alleles) can alter the degree of drug interaction observed upon co-administration of inhibitors. Likewise, there are reports of genotype-dependent interactions with CYP2C19 substrates. For example, fluvoxamine, an inhibitor of CYP2C19, increased exposure to lansoprazole by 3.8-fold in homozygous EMs and by 2.5-fold in heterozygous EMs, whereas no difference in any pharmacokinetic parameters was found in PMs [62].

Other DMEs, such as CYP2C9 or CYP2C19, have not been studied as thoroughly as CYP2D6 in terms of rates of phenoconversion, but the prevalence of the use of interacting drugs provides a likely estimate of the problem.

A comprehensive literature search by He *et al*. [63] identified 32 drugs that are subject to CYP2C9-mediated polymorphic metabolism. Of these, drugs that are subject to clinically relevant polymorphic metabolism with clinical significance include *S*-warfarin, phenytoin, a number of angiotensin II receptor blockers and sulfonylurea oral hypoglycaemic drugs [44]. A retrospective study of 6772 warfarin-treated in-patients in a university hospital in Finland reported that a total of 48% of warfarin-treated in-patients were exposed to interacting co-medication [64]. In this study, the adjusted odds ratio (OR) for bleeding was highest for users of CYP2C9 inhibitors [OR 3.6, 95% confidence interval (CI) 2.4–5.6] followed by cyclo-oxygenase-2-selective ('coxibs') and cyclo-oxygenase-2-nonselective nonsteroidal anti-inflammatory drugs (NSAIDs; OR 3.1, 95% CI 1.4–6.7 and OR 2.6, 95% CI 1.6–4.2, respectively). While not all NSAIDs induce phenoconversion to CYP2C9 PM phenotype, their concurrent use (with warfarin) may induce bleeding, a clinical outcome of interest ('pharmacodynamic phenoconversion') in warfarin association studies, potentially with the same adverse impact on the conclusions reached from these studies. Amiodarone is one of the inhibitors of CYP2C9, and from the data reported by Klein *et al*. [65] it appears that co-administration of amiodarone with warfarin could reduce the genotype-determined weekly maintenance dose of warfarin by as much as 20–35%, depending on the genotype of the patient. A retrospective, cross-sectional population-based register study of patients being dispensed warfarin reported that co-medication with amiodarone was associated with a significant decrease of 8.2% in the dispensed dose of warfarin [66]. In the same study, co-medication with carbamazepine warranted a massive 40% increase in the dispensed dose of warfarin because of the CYP2C9 enzyme-inducing effect of carbamazepine. In a more recent study by Santos *et al*. [67], 111 (12.8%) of the 866 patients maintained on warfarin were concurrently taking amiodarone, and the maintenance dose of warfarin was significantly lower (by 19.6%) in these patients compared with the 755 who were not on amiodarone. The frequency of patients whose international normalized ratio (INR) was within the therapeutic range was 51.4% in the users and 60.5% in the non-users of amiodarone. In a warfarin pharmacogenetic association study reported by Wadelius *et al*. [68], 46% of the 1496 genotyped patients were receiving drugs potentiating, and 3.7% receiving drugs that decrease, warfarin effect (as reflected in INR).

Table 5

Examples of potential clinical consequences of phenoconversion

Phenoconversion of CYP isoform	Examples of potential consequences of phenoconversion Increased toxicity of parent drug and/or decreased effect of the prodrugs
CYP2C9	Increased sensitivity to warfarin Phenytoin-induced neurotoxicity Candesartan-induced hypotension Decreased efficacy of losartan? Sulphonylurea-induced hypoglycaemia?
CYP2C19	Decreased therapeutic effect of clopidogrel Diminished antimyeloma efficacy of thalidomide Decreased likelihood of antiviral response to nelfinavir
CYP2D6	Decreased analgesic effect from codeine, tramadol and oxycodone Perhexiline-induced toxicity (e.g. neuropathy) Decreased therapeutic effect of tamoxifen Extrapyramidal symptoms from perphenazine Increased β-blockade from metoprolol Increased risk of dizziness from carvedilol

[Compiled from literature review.]

Phenoconversion, clinical outcomes and personalized medicine

Table 5 provides some examples of potential clinical consequences of phenoconversion.

There is a strong mechanistic support for an association between *CYP2D6* genotype and tamoxifen efficacy [69, 70]. Nevertheless, studies investigating the association between *CYP2D6* genotype and clinical outcomes following treatment with tamoxifen for breast cancer have yielded conflicting evidence [69, 71–74]. Kiyotani *et al*. [75] reported an important observation that in breast cancer patients who received tamoxifen therapy in combination with other therapies, there was no significant association between *CYP2D6* genotype and recurrence-free survival. However, a subgroup analysis restricted to those who received only tamoxifen monotherapy revealed a positive association. Although there are a number of factors that may explain these inconsistent findings for what may (or may not) be a true association, mechanistic pharmacokinetic considerations alone strongly suggest that phenoconversion may be one factor. One study has reported that following absolute increases of 25, 50 and 75% in the proportion of time on tamoxifen with overlapping use of paroxetine, there were 24, 54 and 91% increases in the risk of death from breast cancer, respectively. In contrast, no such risk was observed with other SSRI antidepressants, although the number of women taking these, especially fluoxetine, was relatively smaller [76].

At least one study suggests that the risk of an adverse clinical outcome following warfarin therapy may be greater following phenoconversion than it is with having a variant *CYP2C9* allele. From their prospective observational study of 115 patients, Gschwind *et al*. [77] reported that in terms of the risk of overanticoagulation, hazard ratios were 2.8 in the presence of a CYP2C9 inhibitor and 1.7 in the presence of *CYP2C9* polymorphisms. They also found that the presence of *CYP2C9* polymorphisms almost tripled the risk of overanticoagulation (hazard ratio 2.91) in the presence of a clinically significant drug–drug interaction.

In a large study of warfarin-exposed patients, the incidence of first-time severe bleeding was 2.3 per 100 patient-years [78] but male gender and use of drugs potentially interacting with warfarin were the only independent risk factors of severe bleeding, with hazard ratios of 2.8 and 2.3, respectively. Gasse *et al*. [79] have also reported that 58% of study-eligible patients used potentially interacting drugs during continuous warfarin treatment, which was associated with a 3- to 4.5-fold increased risk of serious bleeding in long-term warfarin users. While it is acknowledged that not all interacting drugs induce a DME phenoconversion, many of these nonphenoconverting drugs have a pharmacodynamic effect on the clinical outcome of interest (bleeding in the case of warfarin), thereby further prejudicing the conclusions from association studies. Amiodarone inhibits CYP2C9, and Lam *et al*. [80] reported that overall, 56 (0.8%) amiodarone recipients and 23 (0.3%) control patients receiving warfarin were hospitalized for haemorrhage within 30 days of initiating amiodarone (adjusted hazard ratio 2.45, 95% CI 1.49–4.02). Seven of 56 (12.5%) patients hospitalized for a haemorrhage after starting amiodarone died in hospital.

Ma *et al*. [81] have summarized and highlighted the conflicting evidence from larger, more recent studies that investigated the association between clinical outcomes following clopidogrel therapy and *CYP2C19* genotype or use of proton-pump inhibitors (PPIs). A meta-analysis of data accumulated from 15 large association studies also did not indicate a substantial or consistent influence of *CYP2C19* gene polymorphisms on clinical outcome [82]. Similar controversy surrounds the concurrent use of clopidogrel with omeprazole [81]. Some studies have reported adverse clinical outcomes following concurrent use of a PPI and clopidogrel compared with clopidogrel alone [83–85]. Other studies have failed to confirm this observation [86–89]. Whether or not concurrent use of clopidogrel with a PPI adversely affects the clinical outcome remains unresolved at present, but the precautionary principle requires that until the issue is resolved, association studies should adjust for the use of CYP2C19 inhibitors that may induce phenoconversion. This being so, the study reported by Shrestha *et al*. [90] must raise some concerns in the context of association studies with clopidogrel. Their study of 60 patients who were on clopidogrel revealed that 39 (65%) of these had a

co-prescription of (any) PPI for a mean 203 days. One patient on clopidogrel was receiving a CYP2C19 inhibitor in addition to a PPI. Of course, not all PPIs have the same CYP2C19 phenoconversion potential; omeprazole is reportedly the most potent [91].

Phenoconversion deserves serious attention

Genotype–phenotype association studies

The implications of this potential genotype–phenotype mismatch regarding a DME in association studies that aim to correlate only the genotype with clinical outcomes with a view to promoting personalized medicine are self-evident.

Large-scale prospective studies that have investigated associations such as genotypes of *CYP2C9* with efficacy and bleeding following warfarin therapy, *CYP2D6* and survival following tamoxifen therapy, *CYP2C19* and cardiovascular events following clopidogrel therapy and *UGT1A1* and myelotoxicity following irinotecan therapy have yielded inconsistent and often conflicting evidence on the strength of the association studied. There may be a number of potential explanations to account for this. For example, with regard to the debate on the role of *CYP2D6* genotype and treatment outcome following tamoxifen therapy in patients with early postmenopausal oestrogen receptor-positive breast cancer, Brauch and Schwab [92] have summarized the available pharmacokinetic, pharmacodynamic and pharmacogenetic evidence with a view to resolving the controversy based on the recognized methodological and statistical issues. They concluded that cumulative evidence suggests that genotyping for *CYP2D6* is clinically relevant in postmenopausal women. In subsequent correspondence, they emphasized that although published data with regard to the interactions of CYP2D6 inhibitors with tamoxifen may appear inconclusive, attention should be paid to differences in relevant study sizes and designs, which may prohibit direct study comparisons, and they endorse the recommendations by academic societies and regulatory agencies to exercise caution in co-administering CYP2D6 inhibitors when using tamoxifen [93].

Therefore, a question inevitably arises as to whether the conflicting results observed in association studies may be due, at least in part, to concurrent medications that elicit a PM phenotype. The controversy concerning the clinical relevance of co-administration of CYP2D6 inhibitors with tamoxifen or CYP2C19 inhibitors with clopidogrel will also continue despite a strong mechanistic basis to support an adverse outcome following such concurrent therapy, but this should not, for the time being, distract from considering drug-induced phenoconversion as a phenomenon that needs to be addressed in genotype–clinical outcome association studies if the laudable aims of personalized medicine are to be realized. Ratain *et al*. [70] have recently proposed a number of quality metrics for pharmacogenetic association studies, and taking account of concomitant drugs is one of the 10 elements they propose.

Routine clinical practice

The risks from phenoconversion are not limited to the analysis of association studies. They persist well into the future, even after an accurate genotype-based dose has been determined, because of the potential risk that a phenoconverting drug may be (inadvertently) co-prescribed in the future. Concurrent use of high-risk antimicrobials with the associated risk of excess bleeding has been reported to be high (42·6%) among warfarin users [94]. In a study which determined an incidence rate for an INR $\geq$ 6 with concurrent use of antimicrobials reported this rate to be 6.9 per 10 000 treatment days overall; sulfamethoxazole combined with trimethorprim (a potent CYP2C9 inhibitor) most strongly increased the risk of overanticoagulation, with an adjusted relative risk of 20.1 (95% CI 10.7–37.9) [95]. Another illustration of an adverse consequence from a change in the pattern of clinical use of drugs even after a genotype-based dose has been carefully selected is the experience with perhexiline in Australia. Perhexiline is an effective anti-anginal drug metabolized by CYP2D6. As a result of its toxicity (hepatotoxicity and neuropathy related to *CYP2D6* polymorphism), it was removed from the market worldwide except Australia where it continues to be available for clinical use provided the patients are *CYP2D6* genotyped pretreatment and a genotype-specific dose prescribed. While this strategy has worked extremely well, there are isolated cases where patients doing well later developed toxic concentrations of or toxicity to perhexiline following a co-prescription of not only strong but also moderate CYP2D6 inhibitors [96, 97]. This underscores how, even when a personalized drug and its dose have been selected, careful vigilance is required for the consequences of unintentional phenoconversion.

Need for a revised nomenclature

In the past 30 years, pharmacogenetics has acquired its own distinctive vocabulary and associated acronyms to describe DME phenotypes. We believe that it is timely to reconsider these and suggest some revisions, particularly in respect of the various pharmacogenetic drug oxidation polymorphisms that have been described over the past few decades.

Before considering these revisions, it is helpful to trace the historical origins of the acronyms currently in use. In 1975, Eichelbaum *et al*. [98] described a few individuals who were unable to mediate the putative *N*-oxidation

of the alkaloid sparteine, whom they termed the 'nonmetabolizers' to distinguish them from their counterparts, the 'metabolizers'. This appears to be the first recorded instance of the use of these two terms. Subsequently, in their paper describing the genetically determined polymorphic hydroxylation of the antihypertensive drug debrisoquine in man, Mahgoub *et al*. [99] introduced the terms 'extensive metabolizers' and retained the term 'nonmetabolizers', with the corresponding acronyms EM and NM respectively, to define the two phenotypes. In their abstract entitled 'Defective alicyclic hydroxylation of debrisoquine in man' to the British Pharmacological Society, Angelo *et al*. [100] used the acronyms EM and NM to describe the two phenotypes. However, the Editor of the Journal, quite correctly, changed the NM acronym to PM for 'poor metabolizers' because the use of the term 'nonmetabolizer' and its associated acronym was not considered strictly appropriate because the so-called nonmetabolizers did hydroxylate debrisoquine, albeit to a negligible extent. In their two subsequent publications describing the pharmacogenetic basis of the interindividual differences in the oxidative metabolism of sparteine, Eichelbaum *et al*. [101, 102] continued to use the terms 'metabolizer' and 'nonmetabolizer' to describe the two phenotypes. In 1982, they too adopted the acronyms EM and PM [103], and the two acronyms EM and PM have now been in universal use for more than 30 years. Two additional acronyms, namely IM and UM, have also appeared since to describe individuals characterized as 'intermediate' and 'ultrarapid' metabolizers, respectively. Despite extensive literature search, the first study we could locate that used these two additional acronyms (UM and IM) in a single publication was published in 1997 [104]. Extremely rapid hydroxylation appears to have been first described in 1985 by Bertilsson *et al*. [105], who continued to use the term 'ultrarapid hydroxylators' until 1993 [106]. Later that year, Johansson *et al*. [107] introduced the term 'ultrarapid metabolizers' to describe these individuals. In 1995, the term 'ultrarapid metabolizers' was formalized to designate a defined subgroup of the population [108]; however, none of these reports introduced the acronym UM. In 1995, Nordin and Bertilsson reverted to using the term 'rapid hydroxylators' (again without an acronym) in their review of the active hydroxy-metabolites of antidepressants [109]. In 1993, Mura *et al*. [110] confirmed that in EM subjects, part of the phenotypic heterogeneity could be explained by a subgroup of individuals heterozygous for a mutant allele. It was not until 2000 that Raimundo *et al*. [111] identified a polymorphic mutation within *CYP2D6*2* alleles, with a frequency of 20% in the general population, that allowed establishment of a genotype for the identification of >60% of IMs in Caucasian populations. It has also become apparent that resulting from the effects of drug–drug and drug–disease interactions, the phenotype may change despite the immutability of the genotype (phenoconversion).

It seems timely, therefore, to reconsider the utility of these acronyms as currently used and whether they should perhaps be redesignated. The major reason for consideration of a reconfiguration arises from the use of the terms EM and PM to describe both the genotype and the phenotype, which can give rise to misinterpretations, as we have discussed above. Numerous drugs are known to inhibit DMEs, in particular CYP2D6, thereby converting the phenotype of a subject from EM to a transient PM status. There is also the possibility that through enzyme induction, a phenotypic PM could be converted to a phenocopy transient EM. However, this possibility is not very common because if a DME genetic variant is so damaging that it results in an enzyme with little or no activity, then it is most unlikely for a co-medicated drug to convert a nonfunctioning enzyme to a functional state.

In a partial attempt to resolve this genotype–phenotype mismatch, Hicks *et al*. [10] have proposed the use of the prefix 'g' to describe genotypic categorization of subjects and introduced the acronyms 'gUM', 'gEM', 'gIM' and 'gPM'. We would suggest developing this concept further, by adding the prefix 'p', to recognize the distinction between true genotypic categories who behave as predicted by their genotype and their phenotypic PM counterparts resulting from phenoconversion. We, therefore, suggest the following additional acronyms to describe phenoconverted individuals, namely the terms 'pUM', 'pEM', 'pIM' and 'pPM' for those in whom there is a clearly defined metabolic phenotype basis for these (phenoconversion-induced) categorizations, regardless of their genotype. For example, in association studies and in clinical settings, gEM or gIM individuals in receipt of a potent CYP2D6 inhibitor would be classified as pPM individuals, who would be predicted to respond as (and therefore be grouped with) genotypic PM (gPM) individuals. This may not be the complete solution, but it is a major step forward given the current (exclusively and unsatisfactory) genotype-based approaches to analysing association studies. As argued by us in this review, we believe that it is important to make this distinction, particularly in respect of the interpretation of the outcomes of clinical studies purporting to investigate the relationship between drug metabolism genotype and drug response as well as during the clinical use of multiple drugs where, unwittingly, phenoconversion is known to occur.

Addressing phenoconversion in future studies

Phenoconversion has significant implications for the conduct and analysis of genotype-based clinical association studies, and if not taken fully into account, may compromise their interpretation. A number of pharmacogenetic-guided warfarin dosing algorithms do take into account amiodarone as a variable to be factored

in, but given the number of drugs that inhibit CYP2C9, a question arises as to whether adjusting for the use of only one CYP2C9-inhibiting drug is sufficient. While most studies have reported concurrent use of amiodarone by warfarin-treated patients to be in the order of ~6% at the most, Wadelius *et al*. [68] reported the use of drugs potentiating the anticoagulant effect of warfarin in 46% of their cohort.

Studies on tamoxifen in breast cancer patients provide an even more compelling case for preferring phenotyping over genotyping in association studies. A large number of trials that evaluated an association between *CYP2D6* genotype and response to tamoxifen therapy have been completed, and these have yielded inconsistent results. Reviews of these published studies have identified a number of factors to account for this, including the failure to adjust for the concomitant use of CYP2D6 inhibitors [69, 112]. In one study of 40 patients on tamoxifen treatment, the dextromethorphan phenotyping test was demonstrated to be more reliable in predicting plasma concentrations of endoxifen, the pharmacologically active metabolite of tamoxifen. This was strikingly demonstrated in a patient who was also receiving paroxetine. Genotyping alone would not have predicted the low observed concentrations of endoxifen in this patient [113]. In another study of 97 patients on tamoxifen therapy, CYP2D6 phenotype (using dextromethorphan as the probe) showed a stronger association with plasma endoxifen concentrations than did *CYP2D6* genotype. Furthermore, in this study, phenotype accounted for 26%, whereas genotype accounted for only 12%, of the variability in plasma endoxifen levels [114]. Clinically, the ^{13}C-dextromethorphan breath test has already been used successfully in a study of 65 patients to predict endoxifen levels in breast cancer patients treated with tamoxifen [115]. Positive and negative predictive values for the suggested threshold serum level of endoxifen (5.97 ng ml^{-1}) for breast cancer recurrence rate were 100 and 90%, respectively, for both CYP2D6 phenotype (by dextromethorphan breath test) and genotype (*CYP2D6* gene activity score of 1.0). These investigators concluded that along with *CYP2D6* genotyping, the dextromethorphan breath test might be of value in selection of individualized therapy, especially when there is a likelihood of concomitant use of CYP2D6-inhibiting medication that alters the phenotype. These findings spell optimism for future studies, which are expected to aim at associating DME phenotype when investigating outcomes following tamoxifen therapy [116, 117].

While genotyping categorizes patients into discrete genotype-based groups, phenotyping provides more personalized intragenotypic information relevant to personalized therapy. Despite its significance with regard to clinical outcomes, phenotyping has not enjoyed the same appeal as has genotyping among the investigators of genotype–phenotype associations and personalized medicine. These association studies should pay special attention to the phenomenon of phenoconversion in their cohorts, especially if the results of these studies are likely to have a major impact on public health policies promoting personalized medicine and payer attitudes to reimbursement. For analysis and interpretation of association studies, genotypic EMs who are phenotypic PMs should be pooled with genotypic PMs. Two critical issues with regard to phenotyping of subjects in genotype–phenotype association studies are the phenotyping protocols and the timing of phenotyping of study subjects.

Without abandoning genotyping, phenotyping of subjects in genotype-based association studies should be routine. Clearly, therefore, what is required is the availability of a rapid phenotyping test that could be performed safely, effectively, cheaply and rapidly to compute a parameter that would be highly correlated with the clearance (and thus, dose requirement) of the medication to be studied or prescribed.

Phenotyping protocols

A whole range of metabolic probe drugs have been widely used in early pharmacology and pharmacogenetic studies and proposed with regard to phenotyping procedures. For example, for determining CYP2D6 phenotype, investigators have used debrisoquine, sparteine, dextromethorphan, metoprolol, risperidone, tramadol and venlafaxine. Losartan, tolbutamide and flurbiprofen have been used for determining CYP2C9 metabolic phenotype, whereas omeprazole, lansoprazole, proguanil and *S*-mephenytoin are frequently used for measuring CYP2C19 activity.

With regard to CYP2D6 phenotype, Bozkurt *et al*. [118] have shown that CYP2D6 phenotype status determined by a 10 mg debrisoquine tablet, a 100 mg sparteine tablet, a 20 mg dextromethorphan capsule or a 100 mg metoprolol tablet orally are all highly correlated. The dextromethorphan metabolic ratio (DMR) seems independent of its ingested dose across a range of doses [119], and a moderate dose, typically 20–30 mg, is just as efficient. Frank *et al*. [120] compiled a list of criteria useful when selecting the best metrics to reflect CYP2D6 metabolic activity. Following a comprehensive search of the literature on CYP2D6 phenotyping studies, they concluded that dextromethorphan and debrisoquine are the best CYP2D6 phenotyping drugs, with debrisoquine having the problem of very limited availability as a therapeutic drug. In most cases, however, dextromethorphan is the preferred probe due to its wide safety margin and availability. The DMR, typically determined from a urine sample collected over 0–8 h postdose following the ingestion of dextromethorphan, is well established as a marker of CYP2D6 metabolizer status. With regard to the phenotyping protocol, the key issues are the choice of the analyte and the assay technique. Given the susceptibility of urinary metoprolol or dextromethorphan metabolic

ratio to changes in urinary pH [121], alternative *in vivo* markers of CYP2D6 with low susceptibility to changes in urinary pH, such as point estimates from plasma concentrations or breath test, are to be highly preferred. Hu *et al*. [122] have shown that the plasma sample at 2, 3, 4 or 5 h or the saliva sample at 6 h could be used for determining the DMR. In one study in healthy Caucasian subjects, the DMR from urine collected over the time interval 0–4 h after the dose correlated reasonably well with those determined from plasma concentrations obtained at 3 h [123]. More recently, it has been shown that the DMRs determined using 0–8 h urine collection or a serum sample at 3 h postdose also correlate well with each other [124]. There is also available a ^{13}C-dextromethorphan breath test. Leeder *et al*. [125] have shown this test to be highly reliable in suitable test conditions, and it has been used successfully in the clinical setting [115].

Several probes of CYP2C9 activity have been suggested. These include *S*-flurbiprofen (4′-hydroxylation), *S*-warfarin (7-hydroxylation), tolbutamide (methylhydroxylation), phenytoin (4′-hydroxylation), losartan (oxidation to form E-3174) and diclofenac (4′-hydroxylation). For most of these, little information exists regarding their use, largely due to the narrow therapeutic index of some. Mephenytoin has long been considered the standard CYP2C19 phenotyping probe, but in many countries racemic mephenytoin can no longer be administered to humans, and problems such as sample stability and adverse effects have prompted the investigation of potential alternatives, such as omeprazole, which is metabolized to two major metabolites, 5-hydroxyomeprazole (CYP2C19) and omeprazole sulfone (CYP3A4). Balian *et al*. [126] have used single oral doses of omeprazole (20 mg) and mephenytoin (100 mg) as probes for the CYP2C19 metabolic activity and concluded that omeprazole hydroxylation as measured using the ratio of omeprazole to 5′-hydroxyomeprazole in serum 2 h after dosing separated EM and PM phenotypes with complete concordance. Other studies have used a 3 h plasma sample with equal reliability. At present, (*R*)-omeprazole plasma metabolic ratio at 4 h has been shown to discriminate the three CYP2C19 genotype groups better than does (*S*)-omeprazole or racemic omeprazole metabolic ratio [127]. More recently, an even simpler ^{13}C-pantoprazole breath test has been developed for determining CYP2C19 phenotype [128], which is able to detect effectively the subjects who are phenotypic CYP2C19 PMs [129].

The last decade has also witnessed an increasing interest in the use of cocktail approaches to determine the metabolic phenotype of a number of DMEs simultaneously; these include cocktails such as Basel, CEIBA, Cooperstown, Geneva, Inje, Karolinska and Pittsburgh. These cocktails typically determine the metabolic phenotypes of a combination of multiple CYP isoforms, such as CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP3A4 and CYP2E1, and NAT2 or xanthine oxidase. While they have been helpful in studying the genetically determined polymorphic profile of various populations and in drug interaction studies, their utility in genotype–phenotype association studies remains to be established, especially with regard to phenotyping protocols, simplicity and assay techniques. In principle at least, they ought to be useful in studying genotype–DME phenotype mismatch, because most drugs are metabolized by multiple forms of CYP isoforms.

The phenotyping protocols described above require administration of a metabolic probe drug, an intervention that could conceivably be viewed as invasive. These protocols also require sophisticated assay technologies.

Search for an endogenous phenotyping probe

As discussed, recent years have seen a proliferation in the unravelling of the genetic basis and the allelic variability of the *CYP2D6* locus, and it is clear that it remains difficult to translate allelic composition into phenotype status, and this has been made even more difficult by drug-induced phenoconversion. There remains, therefore, a strong case for developing a simple and preferably non-invasive functional test to assess the metabolic phenotype. This invariably means the search for an appropriate endogenous substrate whose metabolism is polymorphic.

Recently, there is a great interest in the role of CYP2D6 in human brain. Epidemiological studies have suggested its association with the incidence and prevalence of a number of central nervous system diseases, essentially related to neurotransmitter dysfunction. Human CYP2D6 is involved in the metabolism of various neurotransmitters and neurosteroids [130, 131]. The neurotransmitters that have attracted the greatest interest to date are dopamine and serotonin [132]. In the case of a test for CYP2D6 (where the structural requirements for a substrate are now clearly defined), this calls for looking at a range of endogenous basic nitrogenous compounds. This opens up exciting avenues for future research concerning whether it may be possible to phenotype a subject for CYP2D6 activity by an assay of the end-product(s) of these neurotransmitters in the urine, possibly by a simple non-interventional direct test. Most promising among these might be endogenous amines, such as 5-methoxytryptamine and 5-methoxy-*N*,*N*-dimethyltryptamine, which are suggested to be metabolized by CYP2D6 [133, 134]. Availability of such a test would not only transform the conduct and analysis of genotype–phenotype association studies but also greatly facilitate safer prescribing in routine clinical practice.

Timing of phenotyping

Given that prospective association studies have a relatively long duration, a question arises as to when the study subjects should be phenotyped. It is worth bearing in mind that unlike the genotype, the DME phenotype of a subject is dynamic and can change from week to week, depending on the pattern (frequency and duration) of co-medication

use during the study period. Although the timing of phenotyping will therefore be guided by the circumstance of each subject, the subjects should ideally be phenotyped at enrolment and also in close temporal relationship to the clinical outcome of interest. Other alternatives are to phenotype each subject at least two or three times randomly during the study or every time there is a change in co-medication use. Phenotyping tests based on the breath test can measure individual DME activity relatively non-invasively and easily within a short period of time (30–50 min), permitting their use as often as considered necessary.

Conclusions

It is clear that there are many practical issues to be resolved before 'personalized medicine', evolving from genotype-based analysis of relevant DMEs, can be implemented into clinical practice. Principal issues arise from phenoconversion as a result of co-medications and comorbidities. Consequently, the genotype of a patient provides only a fraction of the information required to guide the choice of 'the right drug at the right dose the first time'.

In genotype-based personalized medicine, potentially the simplest situation is identifying a different dose regimen for patients with the UM, EM and PM genotypes. Genotyping procedures are sufficiently widely available to allow assessment of a patient's genotype. However, the data on the patient's genotype needs to be supplemented by clinical evidence of the efficacy and safety of genotype-specific dosage regimens. At present, such data are rarely available, and the recommendations on genotype-specific doses are generally empirical. Both the Clinical Pharmacogenetics Implementation Consortium and the Dutch Pharmacogenetics Working Group have produced guidelines on genotype-specific doses for a number of drugs based on available strands of evidence. However, there is no information on how widely these guidelines are used clinically, and importantly, these 'evidence-based' guidelines have not been tested in prospective clinical trials for their 'clinical effectiveness' in personalizing medicine in terms of improved clinical outcomes. In the context of the guideline on genotype-based warfarin therapy, the divergent and clinically questionable results from the three recently published large studies on genotype-guided warfarin therapy, based on surrogate end-points of INR in the therapeutic range, may explain the clinical reservations on genotype-guided therapy [135–137]. Closely related to the question of costs and reimbursement is the all important payer perspective which is quite rightly concerned with genotype-determined reduction of clinically relevant risk in absolute terms and not in relative terms (absolute *vs*. relative risk reduction).

However, the above situation is relatively simple in comparison to the challenges of developing 'personalized medicine' against the clinical background of polypharmacy, drug interactions and the potential for phenoconversion discussed in this paper. If the size of the population with genotype–phenotype mismatch is as large as it appears to be, there is a real risk that studies that focus only on the DME genotype, correlating it with clinical outcomes, may miss clinically relevant pharmacogenetic associations, thus compromising any potential for advancing the prospects of personalized medicine. Whether it may or may not be possible to develop prediction algorithms for the effect of particular drugs on metabolism once the genotype of a patient is known remains to be seen.

While earlier phenotyping tests presented challenges in terms of the accuracy of urine collections and the susceptibility of phenotype determination to urinary pH, much simpler and more reliable tests are now available. Phenotyping tests based on single time point plasma concentrations or breath tests using dextromethorphan and omeprazole or pantoprazole for determination of CYP2D6 and CYP2C19 phenotype, respectively, have the attractions of relative simplicity, safety and expediency of use in genotype–phenotype association studies. These phenotyping procedures are much less resource intensive and can be incorporated readily into the protocols of genotype–clinical outcome association studies.

The practical implications of phenoconversion are enormous. In the context of genotype-based association studies and personalized medicine, two options present themselves; one is to undertake a subgroup analysis of outcomes in genotypic EM patients by their DME-inhibiting co-medicines and the other is to check the phenotype of a reasonably sized subset of genotypic EMs to estimate the rates of genotype–phenotype mismatch. Even with strong and reliable data associating patients' genotypes with clinical outcome(s), there are problems in translating this knowledge clinically into usable information during routine pharmacotherapy because of drug interactions which remain a major clinical and public health issue. Principal concerns that would need to be addressed thoroughly in the future include the identification of the following factors: (i) drugs that are susceptible to phenoconversion; (ii) co-medications that can cause phenoconversion; and (iii) dosage amendments that need to be applied during and following phenoconversion. The resolution of these concerns presents many challenges.

Competing Interests

Both authors have completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare: no support from any organization for the

submitted work; no financial relationships with any organizations that might have an interest in the submitted work in the previous 3 years; no other relationships or activities that could appear to have influenced the submitted work.

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REFERENCES

1 Brockmöller J, Kirchheiner J, Meisel C, Roots I. Pharmacogenetic diagnostics of cytochrome P450 polymorphisms in clinical drug development and in drug treatment. Pharmacogenomics 2000; 1: 125–51.

2 Sim SC, Risinger C, Dahl ML, Aklillu E, Christensen M, Bertilsson L, Ingelman-Sundberg M. A common novel CYP2C19 gene variant causes ultrarapid drug metabolism relevant for the drug response to proton pump inhibitors and antidepressants. Clin Pharmacol Ther 2006; 79: 103–13.

3 Kirchheiner J, Bertilsson L, Bruus H, Wolff A, Roots I, Bauer M. Individualized medicine – implementation of pharmacogenetic diagnostics in antidepressant drug treatment of major depressive disorders. Pharmacopsychiatry 2003; 36 (Suppl. 3): S235–43.

4 Solus JF, Arietta BJ, Harris JR, Sexton DP, Steward JQ, McMunn C, Ihrie P, Mehall JM, Edwards TL, Dawson EP. Genetic variations in eleven phase I drug metabolism genes in an ethnically diverse population. Pharmacogenomics 2004; 5: 895–931.

5 Ozawa S, Soyama A, Saeki M, Fukushima-Uesaka H, Itoda M, Koyano S, Sai K, Ohno Y, Saito Y, Sawada J. Ethnic differences in genetic polymorphisms of CYP2D6, CYP2C19, CYP3As and MDR1/ABCB1. Drug Metab Pharmacokinet 2004; 19: 83–95.

6 Kirchheiner J, Nickchen K, Bauer M, Wong ML, Licinio J, Roots I, Brockmöller J. Pharmacogenetics of antidepressants and antipsychotics: the contribution of allelic variations to the phenotype of drug response. Mol Psychiatry 2004; 9: 442–73.

7 Dutch Pharmacogenetics Working Group. Genotype to phenotype translation 'CYP2D6 Genotype to Phenotype'. Available at http://www.pharmgkb.org/page/dpwg (last accessed 23 April 2014).

8 Gaedigk A, Simon SD, Pearce RE, Bradford LD, Kennedy MJ, Leeder JS. The CYP2D6 activity score: translating genotype information into a qualitative measure of phenotype. Clin Pharmacol Ther 2008; 83: 234–42.

9 Kirchheiner J. CYP2D6 phenotype prediction from genotype: which system is the best? Clin Pharmacol Ther 2008; 83: 225–7.

10 Hicks JK, Swen JJ, Gaedigk A. Challenges in CYP2D6 phenotype assignment from genotype data: a critical assessment and call for standardization. Curr Drug Metab 2014; 15: 218–32.

11 Wennerholm A, Dandara C, Sayi J, Svensson JO, Abdi YA, Ingelman-Sundberg M, Bertilsson L, Hasler J, Gustafsson LL. The African-specific CYP2D617 allele encodes an enzyme with changed substrate specificity. Clin Pharmacol Ther 2002; 71: 77–88.

12 Bogni A, Monshouwer M, Moscone A, Hidestrand M, Ingelman-Sundberg M, Hartung T, Coecke S. Substrate specific metabolism by polymorphic cytochrome P450 2D6 alleles. Toxicol in Vitro 2005; 19: 621–9.

13 Brøsen K, Gram LF, Haghfelt T, Bertilsson L. Extensive metabolizers of debrisoquine become poor metabolizers during quinidine treatment. Pharmacol Toxicol 1987; 60: 312–4.

14 Ayesh R, Dawling S, Hayler A, Oates NS, Cholerton S, Widdop B, Idle JR, Smith RL. Comparative effects of the diastereoisomers, quinine and quinidine in producing phenocopy debrisoquine poor metabolisers (PMs) in healthy volunteers. Chirality 1991; 3: 14–8.

15 Vormfelde SV, Brockmöller J, Bauer S, Herchenhein P, Kuon J, Meineke I, Roots I, Kirchheiner J. Relative impact of genotype and enzyme induction on the metabolic capacity of CYP2C9 in healthy volunteers. Clin Pharmacol Ther 2009; 86: 54–61.

16 Oshiro C, Thorn CF, Roden DM, Klein TE, Altman RB. KCNH2 pharmacogenomics summary. Pharmacogenet Genomics 2010; 20: 775–7.

17 Shah RR. Pharmacogenetic aspects of drug-induced torsade de pointes: potential tool for improving clinical drug development and prescribing. Drug Saf 2004; 27: 145–72.

18 Marsenic O. Glucose control by the kidney: an emerging target in diabetes. Am J Kidney Dis 2009; 53: 875–83.

19 Weber O, Bischoff H, Schmeck C, Böttcher MF. Cholesteryl ester transfer protein and its inhibition. Cell Mol Life Sci 2010; 67: 3139–49.

20 Division of Clinical Pharmacology Indiana University P450 drug interaction table. Available at http://medicine.iupui.edu/clinpharm/DDIs/table.aspx (last accessed 17 January 2014).

21 Samer CF, Lorenzini KI, Rollason V, Daali Y, Desmeules JA. Applications of CYP450 testing in the clinical setting. Mol Diagn Ther 2013; 17: 165–84.

22 Olesen C, Harbig P, Barat I, Damsgaard EM. Absence of 'over-the-counter' medicinal products in on-line prescription records: a risk factor of overlooking interactions in the elderly. Pharmacoepidemiol Drug Saf 2013; 22: 145–50.

23 Williamson EM. Interactions between herbal and conventional medicines. Expert Opin Drug Saf 2005; 4: 355–78.

24 Hu Z, Yang X, Ho PC, Chan SY, Heng PW, Chan E, Duan W, Koh HL, Zhou S. Herb-drug interactions: a literature review. Drugs 2005; 65: 1239–82.

25 Goldstein LH, Elias M, Ron-Avraham G, Biniaurishvili BZ, Madjar M, Kamargash I, Braunstein R, Berkovitch M, Golik

A. Consumption of herbal remedies and dietary supplements amongst patients hospitalized in medical wards. Br J Clin Pharmacol 2007; 64: 373–80.

26 Tarirai C, Viljoen AM, Hamman JH. Herb-drug pharmacokinetic interactions reviewed. Expert Opin Drug Metab Toxicol 2010; 6: 1515–38.

27 Amchin J, Ereshefsky L, Zarycranski W, Taylor K, Albano D, Klockowski PM. Effect of venlafaxine versus fluoxetine on metabolism of dextromethorphan, a CYP2D6 probe. J Clin Pharmacol 2001; 41: 443–51.

28 Nakashima D, Takama H, Ogasawara Y, Kawakami T, Nishitoba T, Hoshi S, Uchida E, Tanaka H. Effect of cinacalcet hydrochloride, a new calcimimetic agent, on the pharmacokinetics of dextromethorphan: in vitro and clinical studies. J Clin Pharmacol 2007; 47: 1311–9.

29 Pope LE, Khalil MH, Berg JE, Stiles M, Yakatan GJ, Sellers EM. Pharmacokinetics of dextromethorphan after single or multiple dosing in combination with quinidine in extensive and poor metabolizers. J Clin Pharmacol 2004; 44: 1132–42.

30 Laine K, Tybring G, Härtter S, Andersson K, Svensson JO, Widén J, Bertilsson L. Inhibition of cytochrome P4502D6 activity with paroxetine normalizes the ultrarapid metabolizer phenotype as measured by nortriptyline pharmacokinetics and the debrisoquin test. Clin Pharmacol Ther 2001; 70: 327–35.

31 Dalén P, Dahl M, Andersson K, Bertilsson L. Inhibition of debrisoquine hydroxylation with quinidine in subjects with three or more functional CYP2D6 genes. Br J Clin Pharmacol 2000; 49: 180–4.

32 Lam YW, Gaedigk A, Ereshefsky L, Alfaro CL, Simpson J. CYP2D6 inhibition by selective serotonin reuptake inhibitors: analysis of achievable steady-state plasma concentrations and the effect of ultrarapid metabolism at CYP2D6. Pharmacotherapy 2002; 22: 1001–6.

33 O'Neil WM, Gilfix BM, Markoglou N, Di Girolamo A, Tsoukas CM, Wainer IW. Genotype and phenotype of cytochrome P450 2D6 in human immunodeficiency virus-positive patients and patients with acquired immunodeficiency syndrome. Eur J Clin Pharmacol 2000; 56: 231–40.

34 Jones AE, Brown KC, Werner RE, Gotzkowsky K, Gaedigk A, Blake M, Hein DW, van der Horst C, Kashuba AD. Variability in drug metabolizing enzyme activity in HIV-infected patients. Eur J Clin Pharmacol 2010; 66: 475–85.

35 Mitchell SC, Shah RR, Clements DG, Smith RL. Changes in debrisoquine hydroxylation capacity following liver surgery. Hum Exp Toxicol 1994; 13: 537–41.

36 Monek O, Paintaud G, Bechtel Y, Miguet JP, Mantion G, Bechtel PR. Influence of donor and recipient genotypes on CYP2D6 phenotype after liver transplantation: a study of mutations CYP2D6*3 and CYP2D6*4. Eur J Clin Pharmacol 1998; 54: 47–52.

37 Rost KL, Brockmöller J, Esdorn F, Roots I. Phenocopies of poor metabolizers of omeprazole caused by liver disease and drug treatment. J Hepatol 1995; 23: 268–77.

38 Ohnishi A, Murakami S, Akizuki S, Mochizuki J, Echizen H, Takagi I. In vivo metabolic activity of CYP2C19 and CYP3A in relation to CYP2C19 genetic polymorphism in chronic liver disease. J Clin Pharmacol 2005; 45: 1221–9.

39 Williams ML, Bhargava P, Cherrouk I, Marshall JL, Flockhart DA, Wainer IW. A discordance of the cytochrome P450 2C19 genotype and phenotype in patients with advanced cancer. Br J Clin Pharmacol 2000; 49: 485–8.

40 Helsby NA, Lo WY, Sharples K, Riley G, Murray M, Spells K, Dzhelai M, Simpson A, Findlay M. CYP2C19 pharmacogenetics in advanced cancer: compromised function independent of genotype. Br J Cancer 2008; 99: 1251–5.

41 Ito K, Brown HS, Houston JB. Database analyses for the prediction of in vivo drug-drug interactions from in vitro data. Br J Clin Pharmacol 2004; 57: 473–86.

42 Michalets EL. Update: clinically significant cytochrome P-450 drug interactions. Pharmacotherapy 1998; 18: 84–112.

43 Cavallari LH, Jeong H, Bress A. Role of cytochrome P450 genotype in the steps toward personalized drug therapy. Pharmgenomics Pers Med 2011; 4: 123–36.

44 Hirota T, Eguchi S, Ieiri I. Impact of genetic polymorphisms in CYP2C9 and CYP2C19 on the pharmacokinetics of clinically used drugs. Drug Metab Pharmacokinet 2013; 28: 28–37.

45 Lynch T, Price A. The effect of cytochrome P450 metabolism on drug response, interactions, and adverse effects. Am Fam Physician 2007; 76: 391–6.

46 Sindrup SH, Brøsen K. The pharmacogenetics of codeine hypoalgesia. Pharmacogenetics 1995; 5: 335–46.

47 Ozdemir V, Naranjo CA, Herrmann N, Reed K, Sellers EM, Kalow W. Paroxetine potentiates the central nervous system side effects of perphenazine: contribution of cytochrome P4502D6 inhibition in vivo. Clin Pharmacol Ther 1997; 62: 334–47.

48 Committee for Medicinal Products for Human Use (CHMP). Guideline on the use of pharmacogenetic methodologies in the pharmacokinetic evaluation of medicinal products (EMA/CHMP/37646/2009). European Medicines Agency, London, 12 December 2011. Available at http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2012/02/WC500121954.pdf (last accessed 28 February 2014).

49 Zanger UM, Klein K, Thomas M, Rieger JK, Tremmel R, Kandel BA, Klein M, Magdy T. Genetics, epigenetics, and regulation of drug-metabolizing cytochrome P450 enzymes. Clin Pharmacol Ther 2014; 95: 258–61.

50 Lockhart P, Guthrie B. Trends in primary care antidepressant prescribing 1995–2007: a longitudinal population database analysis. Br J Gen Pract 2011; 61: e565–72.

51 Preskorn SH, Shah R, Neff M, Golbeck AL, Choi J. The potential for clinically significant drug-drug interactions involving the CYP 2D6 system: effects with fluoxetine and paroxetine versus sertraline. J Psychiatr Pract 2007; 13: 5–12.

52 Lohmann PL, Rao ML, Ludwig M, Griese EU, Zanger UM, Mörike K, Maier W, Bagli M. Influence of CYP2D6 genotype and medication on the sparteine metabolic ratio of psychiatric patients. Eur J Clin Pharmacol 2001; 57: 289–95. Erratum in: Eur J Clin Pharmacol 2001; 57: 745.

53 Bramer SL, Suri A. Inhibition of CYP2D6 by quinidine and its effects on the metabolism of cilostazol. Clin Pharmacokinet 1999; 37 (Suppl. 2): 41–51.

54 Cai WM, Chen B, Ling SS, Zhang YD. Terbinafine-associated inhibition of dextromethorphan metabolism in Chinese subjects. Br J Clin Pharmacol 2001; 51: 107–8.

55 Caraco Y, Sheller J, Wood AJ. Impact of ethnic origin and quinidine coadministration on codeine's disposition and pharmacodynamic effects. J Pharmacol Exp Ther 1999; 290: 413–22.

56 Kim K, Johnson JA, Derendorf H. Differences in drug pharmacokinetics between East Asians and Caucasians and the role of genetic polymorphisms. J Clin Pharmacol 2004; 44: 1083–105.

57 Dilger K, Greiner B, Fromm MF, Hofmann U, Kroemer HK, Eichelbaum M. Consequences of rifampicin treatment on propafenone disposition in extensive and poor metabolizers of CYP2D6. Pharmacogenetics 1999; 9: 551–9.

58 Shah RR. Clinical pharmacokinetics: current requirements and future perspectives from a regulatory point of view. Xenobiotica 1993; 23: 1159–93.

59 Llerena A, Berecz R, de la Rubia A, Fernández-Salguero P, Dorado P. Effect of thioridazine dosage on the debrisoquine hydroxylation phenotype in psychiatric patients with different CYP2D6 genotypes. Ther Drug Monit 2001; 23: 616–20.

60 Preskorn SH, Kane CP, Lobello K, Nichols AI, Fayyad R, Buckley G, Focht K, Guico-Pabia CJ. Cytochrome P450 2D6 phenoconversion is common in patients being treated for depression: implications for personalized medicine. J Clin Psychiatry 2013; 74: 614–21.

61 Kumar V, Brundage RC, Oetting WS, Leppik IE, Tracy TS. Differential genotype dependent inhibition of CYP2C9 in humans. Drug Metab Dispos 2008; 36: 1242–8.

62 Yasui-Furukori N, Saito M, Uno T, Takahata T, Sugawara K, Tateishi T. Effects of fluvoxamine on lansoprazole pharmacokinetics in relation to CYP2C19 genotypes. J Clin Pharmacol 2004; 44: 1223–9.

63 He SM, Zhou ZW, Li XT, Zhou SF. Clinical drugs undergoing polymorphic metabolism by human cytochrome P450 2C9 and the implication in drug development. Curr Med Chem 2011; 18: 667–713.

64 Hauta-Aho M, Tirkkonen T, Vahlberg T, Laine K. The effect of drug interactions on bleeding risk associated with warfarin therapy in hospitalized patients. Ann Med 2009; 41: 619–28.

65 Klein TE, Altman RB, Eriksson N, Gage BF, Kimmel SE, Lee MT, Limdi NA, Page D, Roden DM, Wagner MJ, Caldwell MD, Johnson JA; for International Warfarin Pharmacogenetics Consortium. Estimation of the warfarin dose with clinical and pharmacogenetic data. N Engl J Med 2009; 360: 753–64.

66 Andersson ML, Lindh JD, Mannheimer B. The impact of interacting drugs on dispensed doses of warfarin in the Swedish population: a novel use of population based drug registers. J Clin Pharmacol 2013; 53: 1322–7.

67 Santos PCJL, Soares RAG, Strunz CMC, Grinberg M, Ferreira JFM, Cesar LAM, Scanavacca M, Krieger JE, Pereira AC. Simultaneous use of amiodarone influences warfarin maintenance dose but is not associated with adverse events. J Manag Care Pharm 2014; 20: 376–81.

68 Wadelius M, Chen LY, Lindh JD, Eriksson N, Ghori MJ, Bumpstead S, Holm L, McGinnis R, Rane A, Deloukas P. The largest prospective warfarin-treated cohort supports genetic forecasting. Blood 2009; 113: 784–92.

69 de Souza JA, Olopade OI. CYP2D6 genotyping and tamoxifen: an unfinished story in the quest for personalized medicine. Semin Oncol 2011; 38: 263–73.

70 Ratain MJ, Nakamura Y, Cox NJ. CYP2D6 genotype and tamoxifen activity: understanding interstudy variability in methodological quality. Clin Pharmacol Ther 2013; 94: 185–7.

71 Kelly CM, Pritchard KI. CYP2D6 genotype as a marker for benefit of adjuvant tamoxifen in postmenopausal women: lessons learned. J Natl Cancer Inst 2012; 104: 427–8.

72 Zeng Z, Liu Y, Liu Z, You J, Chen Z, Wang J, Peng Q, Xie L, Li R, Li S, Qin X. CYP2D6 polymorphisms influence tamoxifen treatment outcomes in breast cancer patients: a meta-analysis. Cancer Chemother Pharmacol 2013; 72: 287–303.

73 Cronin-Fenton DP, Damkier P, Lash TL. Metabolism and transport of tamoxifen in relation to its effectiveness: new perspectives on an ongoing controversy. Future Oncol 2014; 10: 107–22.

74 Markkula A, Hjertberg M, Rose C, Ingvar C, Jernström H. No association found between CYP2D6 genotype and early breast cancer events in tamoxifen-treated patients. Acta Oncol 2014; 53: 195–200.

75 Kiyotani K, Mushiroda T, Hosono N, Tsunoda T, Kubo M, Aki F, Okazaki Y, Hirata K, Takatsuka Y, Okazaki M, Ohsumi S, Yamakawa T, Sasa M, Nakamura Y, Zembutsu H. Lessons for pharmacogenomics studies: association study between CYP2D6 genotype and tamoxifen response. Pharmacogenet Genomics 2010; 20: 565–8.

76 Kelly CM, Juurlink DN, Gomes T, Duong-Hua M, Pritchard KI, Austin PC, Paszat LF. Selective serotonin reuptake inhibitors and breast cancer mortality in women receiving tamoxifen: a population based cohort study. BMJ 2010; 340: c693. doi: 10.1136/bmj.c693.

77 Gschwind L, Rollason V, Boehlen F, Rebsamen M, Combescure C, Grünenwald M, Matthey A, Bonnabry P, Dayer P, Desmeules JA. Impact of CYP2C9 polymorphisms on the vulnerability to pharmacokinetic drug-drug interactions during acenocoumarol treatment. Pharmacogenomics 2013; 14: 745–53.

78 Lindh JD, Holm L, Dahl ML, Alfredsson L, Rane A. Incidence and predictors of severe bleeding during warfarin treatment. J Thromb Thrombolysis 2008; 25: 151–9.

79 Gasse C, Hollowell J, Meier CR, Haefeli WE. Drug interactions and risk of acute bleeding leading to hospitalisation or death in patients with chronic atrial fibrillation treated with warfarin. Thromb Haemost 2005; 94: 537–43.

80 Lam J, Gomes T, Juurlink DN, Mamdani MM, Pullenayegum EM, Kearon C, Spencer FA, Paterson M, Zheng H, Holbrook AM. Hospitalization for hemorrhage among warfarin recipients prescribed amiodarone. Am J Cardiol 2013; 112: 420–3.

81 Ma TK, Lam YY, Tan VP, Yan BP. Variability in response to clopidogrel: how important are pharmacogenetics and drug interactions? Br J Clin Pharmacol 2011; 72: 697–706.

82 Bauer T, Bouman HJ, van Werkum JW, Ford NF, ten Berg JM, Taubert D. Impact of CYP2C19 variant genotypes on clinical efficacy of antiplatelet treatment with clopidogrel: systematic review and meta-analysis. BMJ 2011; 343: d4588. doi: 10.1136/bmj.d4588.

83 Kreutz RP, Stanek EJ, Aubert R, Yao J, Breall JA, Desta Z, Skaar TC, Teagarden JR, Frueh FW, Epstein RS, Flockhart DA. Impact of proton pump inhibitors on the effectiveness of clopidogrel after coronary stent placement: the clopidogrel Medco outcomes study. Pharmacotherapy 2010; 30: 787–96.

84 Ching GG, Li D, Baker WL, Hohl PK, Mather JF, McKay RG, Lundbye JB. Major adverse cardiac events among postpercutaneous coronary intervention patients on clopidogrel and proton pump inhibitors. Conn Med 2012; 76: 205–11.

85 Burkard T, Kaiser CA, Brunner-La Rocca H, Osswald S, Pfisterer ME, Jeger RV, BASKET Investigators. Combined clopidogrel and proton pump inhibitor therapy is associated with higher cardiovascular event rates after percutaneous coronary intervention: a report from the BASKET trial. J Intern Med 2012; 271: 257–63.

86 Bhatt DL, Cryer BL, Contant CF, Cohen M, Lanas A, Schnitzer TJ, Shook TL, Lapuerta P, Goldsmith MA, Laine L, Scirica BM, Murphy SA, Cannon CP, COGENT Investigators. Clopidogrel with or without omeprazole in coronary artery disease. N Engl J Med 2010; 363: 1909–17.

87 Simon T, Steg PG, Gilard M, Blanchard D, Bonello L, Hanssen M, Lardoux H, Coste P, Lefèvre T, Drouet E, Mulak G, Bataille V, Ferrières J, Verstuyft C, Danchin N. Clinical events as a function of proton pump inhibitor use, clopidogrel use, and cytochrome P450 2C19 genotype in a large nationwide cohort of acute myocardial infarction: results from the French Registry of Acute ST-Elevation and Non-ST-Elevation Myocardial Infarction (FAST-MI) registry. Circulation 2011; 123: 474–82.

88 Schmidt M, Johansen MB, Robertson DJ, Maeng M, Kaltoft A, Jensen LO, Tilsted HH, Bøtker HE, Sørensen HT, Baron JA. Concomitant use of clopidogrel and proton pump inhibitors is not associated with major adverse cardiovascular events following coronary stent implantation. Aliment Pharmacol Ther 2012; 35: 165–74.

89 Mas-Morey P, Calderón-Hernanz B, Oliver-Noguera A, Ripoll-Vera T, Vilanova-Boltó M. Frequency and outcomes of concomitant use of proton pump inhibitors and clopidogrel after hospital discharge. Therapie 2013; 68: 113–5.

90 Shrestha K, Hughes JD, Lee YP, Parsons R. The prevalence of co-administration of clopidogrel and proton pump inhibitors. Qual Prim Care 2011; 19: 35–42.

91 Wedemeyer R-S, Blume H. Pharmacokinetic drug interaction profiles of proton pump inhibitors: an update. Drug Saf 2014; 37: 201–11.

92 Brauch H, Schwab M. Prediction of tamoxifen outcome by genetic variation of CYP2D6 in postmenopausal women with early breast cancer. Br J Clin Pharmacol 2014; 77: 695–703.

93 Brauch H, Schwab M. Re: Damkier: CYP2D6 genotyping and tamoxifen in the treatment of postmenopausal breast cancer. Br J Clin Pharmacol 2014; 78: 433–4.

94 Lane MA, Devine ST, McDonald JR. High-risk antimicrobial prescriptions among ambulatory patients on warfarin. J Clin Pharm Ther 2012; 37: 157–60.

95 Visser LE, Penning-van Bees FJ, Kasbergen AA, De Smet PA, Vulto AG, Hofman A, Stricker BH. Overanticoagulation associated with combined use of antibacterial drugs and acenocoumarol or phenprocoumon anticoagulants. Thromb Haemost 2002; 88: 705–10.

96 Alderman CP, Hundertmark JD, Soetratma TW. Interaction of serotonin re-uptake inhibitors with perhexiline. Aust N Z J Psychiatry 1997; 31: 601–3.

97 Sheikh AR, Westley I, Sallustio B, Horowitz JD, Beltrame JF. Interaction of terbinafine (anti-fungal agent) with perhexiline: a case report. Heart Lung Circ 2014; 23: e149–51.

98 Eichelbaum M, Spannbrucker N, Dengler HJ. Lack of N-oxidation of sparteine in certain healthy subjects. Proceedings of the Sixth International Congress of Pharmacology, Helsinki, 20–25 July 1975 (Abstract 1071) Oxford: Pergamon Press Ltd, 1977: p 450.

99 Mahgoub A, Idle JR, Dring LG, Lancaster R, Smith RL. Polymorphic hydroxylation of debrisoquine in man. Lancet 1977; 2: 584–6.

100 Angelo MM, Dring LG, Idle JR, Lancaster R, Mahgoub A, Smith RL. Defective alicyclic hydroxylation of debrisoquine in man. Proceedings of the British Pharmacological Society, Manchester, 7–9 September 1977. Br J Clin Pharmacol 1977; 4: 725P.

101 Eichelbaum M, Spannbrucker N, Dengler HJ. Influence of the defective metabolism of sparteine on its pharmacokinetics. Eur J Clin Pharmacol 1979; 16: 189–94.

102 Eichelbaum M, Spannbrucker N, Steincke B, Dengler HJ. Defective N-oxidation of sparteine in man: a new pharmacogenetic defect. Eur J Clin Pharmacol 1979; 16: 183–7.

103 Park BK, Eichelbaum M, Ohnhaus EE. 6-beta-hydroxycortisol excretion in relation to polymorphic N-oxidation of sparteine. Br J Clin Pharmacol 1982; 13: 737–40.

104 Sachse C, Brockmöller J, Bauer S, Roots I. Cytochrome P450 2D6 variants in a Caucasian population: allele frequencies and phenotypic consequences. Am J Hum Genet 1997; 60: 284–95.

105 Bertilsson L, Aberg-Wistedt A, Gustafsson LL, Nordin C. Extremely rapid hydroxylation of debrisoquine: a case report with implication for treatment with nortriptyline and other tricyclic antidepressants. Ther Drug Monit 1985; 7: 478–80.

106 Bertilsson L, Dahl ML, Sjöqvist F, Aberg-Wistedt A, Humble M, Johansson I, Lundqvist E, Ingelman-Sundberg M. Molecular basis for rational megaprescribing in ultrarapid hydroxylators of debrisoquine. Lancet 1993; 341: 63.

107 Johansson I, Lundqvist E, Bertilsson L, Dahl ML, Sjöqvist F, Ingelman-Sundberg M. Inherited amplification of an active gene in the cytochrome P450 CYP2D locus as a cause of ultrarapid metabolism of debrisoquine. Proc Natl Acad Sci USA 1993; 90: 11825–9.

108 Meyer UA. Pharmacogenetics: the slow, the rapid, and the ultrarapid. Proc Natl Acad Sci USA 1994; 91: 1983–4.

109 Nordin C, Bertilsson L. Active hydroxymetabolites of antidepressants. Emphasis on E-10-hydroxy-nortriptyline. Clin Pharmacokinet 1995; 28: 26–40.

110 Mura C, Panserat S, Vincent-Viry M, Galteau MM, Jacqz-Aigrain E, Krishnamoorthy R. DNA haplotype dependency of debrisoquine 4-hydroxylase (CYP2D6) expression among extensive metabolisers. Hum Genet 1993; 92: 367–72.

111 Raimundo S, Fischer J, Eichelbaum M, Griese EU, Schwab M, Zanger UM. Elucidation of the genetic basis of the common 'intermediate metabolizer' phenotype for drug oxidation by CYP2D6. Pharmacogenetics 2000; 10: 577–81.

112 Hertz DL, McLeod HL, Irvin WJ Jr. Tamoxifen and CYP2D6: a contradiction of data. Oncologist 2012; 17: 620–30.

113 de Graan AJ, Teunissen SF, de Vos FY, Loos WJ, van Schaik RH, de Jongh FE, de Vos AI, van Alphen RJ, van der Holt B, Verweij J, Seynaeve C, Beijnen JH, Mathijssen RH. Dextromethorphan as a phenotyping test to predict endoxifen exposure in patients on tamoxifen treatment. J Clin Oncol 2011; 29: 3240–6.

114 Antunes MV, Linden R, Santos TV, Wallemacq P, Haufroid V, Classen JF, Andreolla H, Costa N, Fontanive TO, Rosa DD. Endoxifen levels and its association with CYP2D6 genotype and phenotype: evaluation of a southern Brazilian population under tamoxifen pharmacotherapy. Ther Drug Monit 2012; 34: 422–31.

115 Opdam FL, Dezentje VO, den Hartigh J, Modak AS, Vree R, Batman E, Smorenburg CH, Nortier JW, Gelderblom H, Guchelaar HJ. The use of the ^{13}C-dextromethorphan breath test for phenotyping CYP2D6 in breast cancer patients using tamoxifen: association with CYP2D6 genotype and serum endoxifen levels. Cancer Chemother Pharmacol 2013; 71: 593–601.

116 Trojan A, Vergopoulos A, Breitenstein U, Seifert B, Rageth C, Joechle W. The discriminatory value of CYP2D6 genotyping in predicting the dextromethorphan/dextrorphan phenotype in women with breast cancer. Breast Care (Basel) 2012; 7: 25–31.

117 Trojan A, Vergopoulos A, Breitenstein U, Tausch C, Seifert B, Joechle W. CYP2D6 phenotype indicative for optimized antiestrogen efficacy associates with outcome in early breast cancer patients. Cancer Chemother Pharmacol 2013; 71: 301–6.

118 Bozkurt A, Basçi NE, Işimer A, Sayal A, Kayaalp SO. Metabolic ratios of four probes of CYP2D6 in Turkish subjects: a cross-over study. Eur J Drug Metab Pharmacokinet 1996; 21: 309–14.

119 Streetman DS, Ellis RE, Nafziger AN, Leeder JS, Gaedigk A, Gotschall R, Kearns GL, Bertino JS Jr. Dose dependency of dextromethorphan for cytochrome P450 (CYP2D6) phenotyping. Clin Pharmacol Ther 1999; 66: 535–41.

120 Frank D, Jaehde U, Fuhr U. Evaluation of probe drugs and pharmacokinetic metrics for CYP2D6 phenotyping. Eur J Clin Pharmacol 2007; 63: 321–33.

121 Ozdemir M, Crewe KH, Tucker GT, Rostami-Hodjegan A. Assessment of in vivo CYP2D6 activity: differential sensitivity of commonly used probes to urine pH. J Clin Pharmacol 2004; 44: 1398–404.

122 Hu OY, Tang HS, Lane HY, Chang WH, Hu TM. Novel single-point plasma or saliva dextromethorphan method for determining CYP2D6 activity. J Pharmacol Exp Ther 1998; 285: 955–60.

123 Chládek J, Zimová G, Beránek M, Martínková J. In-vivo indices of CYP2D6 activity: comparison of dextromethorphan metabolic ratios in 4-h urine and 3-h plasma. Eur J Clin Pharmacol 2000; 56: 651–7.

124 Jurica J, Bartecek R, Zourkova A, Pindurova E, Sulcova A, Kasparek T, Zendulka O. Serum dextromethorphan/dextrorphan metabolic ratio for CYP2D6 phenotyping in clinical practice. J Clin Pharm Ther 2012; 37: 486–90.

125 Leeder JS, Pearce RE, Gaedigk A, Modak A, Rosen DI. Evaluation of a [^{13}C]-dextromethorphan breath test to assess CYP2D6 phenotype. J Clin Pharmacol 2008; 48: 1041–51.

126 Balian JD, Sukhova N, Harris JW, Hewett J, Pickle L, Goldstein JA, Woosley RL, Flockhart DA. The hydroxylation of omeprazole correlates with S-mephenytoin metabolism: a population study. Clin Pharmacol Ther 1995; 57: 662–9.

127 Yamada S, Shiohira H, Yasui-Furukori N, Tateishi T, Akamine Y, Uno T. The (R)-omeprazole hydroxylation index reflects CYP2C19 activity in healthy Japanese volunteers. Eur J Clin Pharmacol 2013; 69: 1423–8.

128 Desta Z, Modak A, Nguyen PD, Lemler SM, Kurogi Y, Li L, Flockhart DA. Rapid identification of the hepatic cytochrome P450 2C19 activity using a novel and noninvasive [^{13}C]-pantoprazole breath test. J Pharmacol Exp Ther 2009; 329: 297–305.

129 Furuta T, Kodaira C, Nishino M, Yamade M, Sugimoto M, Ikuma M, Hishida A, Watanabe H, Umemura K. [^{13}C]-pantoprazole breath test to predict CYP2C19

phenotype and efficacy of a proton pump inhibitor, lansoprazole. Aliment Pharmacol Ther 2009; 30: 294–300.

130 Cheng J, Zhen Y, Miksys S, Beyoğlu D, Krausz KW, Tyndale RF, Yu A, Idle JR, Gonzalez FJ. Potential role of CYP2D6 in the central nervous system. Xenobiotica 2013; 43: 973–84.

131 Wang X, Li J, Dong G, Yue J. The endogenous substrates of brain CYP2D6. Eur J Pharmacol 2013; 724: 211–8.

132 Haduch A, Bromek E, Daniel WA. Role of brain cytochrome P450 (CYP2D) in the metabolism of monoaminergic neurotransmitters. Pharmacol Reports 2013; 65: 1519–28.

133 Yu AM, Idle JR, Herraiz T, Küpfer A, Gonzalez FJ. Screening for endogenous substrates reveals that CYP2D6 is a 5-methoxyindolethylamine O-demethylase. Pharmacogenetics 2003; 13: 307–19.

134 Yu AM, Idle JR, Byrd LG, Krausz KW, Küpfer A, Gonzalez FJ. Regeneration of serotonin from 5-methoxytryptamine by polymorphic human CYP2D6. Pharmacogenetics 2003; 13: 173–81.

135 Kimmel SE, French B, Kasner SE, Johnson JA, Anderson JL, Gage BF, Rosenberg YD, Eby CS, Madigan RA, McBane RB, Abdel-Rahman SZ, Stevens SM, Yale S, Mohler ER 3rd, Fang MC, Shah V, Horenstein RB, Limdi NA, Muldowney JA 3rd, Gujral J, Delafontaine P, Desnick RJ, Ortel TL, Billett HH, Pendleton RC, Geller NL, Halperin JL, Goldhaber SZ, Caldwell MD, Califf RM, Ellenberg JH, COAG Investigators. A pharmacogenetic versus a clinical algorithm for warfarin dosing. N Engl J Med 2013; 369: 2283–93.

136 Pirmohamed M, Burnside G, Eriksson N, Jorgensen AL, Toh CH, Nicholson T, Kesteven P, Christersson C, Wahlström B, Stafberg C, Zhang JE, Leathart JB, Kohnke H, Maitland-van der Zee AH, Williamson PR, Daly AK, Avery P, Kamali F, Wadelius M, EU-PACT Group. A randomized trial of genotype-guided dosing of warfarin. N Engl J Med 2013; 369: 2294–303.

137 Verhoef TI, Ragia G, de Boer A, Barallon R, Kolovou G, Kolovou V, Konstantinides S, Le Cessie S, Maltezos E, van der Meer FJ, Redekop WK, Remkes M, Rosendaal FR, van Schie RM, Tavridou A, Tziakas D, Wadelius M, Manolopoulos VG, Maitland-van der Zee AH, EU-PACT Group. A randomized trial of genotype-guided dosing of acenocoumarol and phenprocoumon. N Engl J Med 2013; 369: 2304–12.

138 Ieiri I, Yamada S, Seto K, Morita T, Kaneda T, Mamiya K, Tashiro N, Higuchi S, Otsubo K. A CYP2D6 phenotype-genotype mismatch in Japanese psychiatric patients. Pharmacopsychiatry 2003; 36: 192–6.

139 Kotlyar M, Brauer LH, Tracy TS, Hatsukami DK, Harris J, Bronars CA, Adson DE. Inhibition of CYP2D6 activity by bupropion. J Clin Psychopharmacol 2005; 25: 226–9.

140 Güzey C, Norström A, Spigset O. Change from the CYP2D6 extensive metabolizer to the poor metabolizer phenotype during treatment with bupropion. Ther Drug Monit 2002; 24: 436–7.

141 Shiran MR, Chowdry J, Rostami-Hodjegan A, Ellis SW, Lennard MS, Iqbal MZ, Lagundoye O, Seivewright N, Tucker GT. A discordance between cytochrome P450 2D6 genotype and phenotype in patients undergoing methadone maintenance treatment. Br J Clin Pharmacol 2003; 56: 220–4.

142 Abdel-Rahman SM, Gotschall RR, Kauffman RE, Leeder JS, Kearns GL. Investigation of terbinafine as a CYP2D6 inhibitor in vivo. Clin Pharmacol Ther 1999; 65: 465–72.

143 Zourková A, Hadasová E. Paroxetine-induced conversion of cytochrome P450 2D6 phenotype and occurence of adverse effects. Gen Physiol Biophys 2003; 22: 103–13.

144 Köhler D, Härtter S, Fuchs K, Sieghart W, Hiemke C. CYP2D6 genotype and phenotyping by determination of dextromethorphan and metabolites in serum of healthy controls and of patients under psychotropic medication. Pharmacogenetics 1997; 7: 453–61.

145 Liston HL, DeVane CL, Boulton DW, Risch SC, Markowitz JS, Goldman J. Differential time course of cytochrome P450 2D6 enzyme inhibition by fluoxetine, sertraline, and paroxetine in healthy volunteers. J Clin Psychopharmacol 2002; 22: 169–73.

146 Madani S, Barilla D, Cramer J, Wang Y, Paul C. Effect of terbinafine on the pharmacokinetics and pharmacodynamics of desipramine in healthy volunteers identified as cytochrome P450 2D6 (CYP2D6) extensive metabolizers. J Clin Pharmacol 2002; 42: 1211–8.

147 Juřica J, Žourková A. Dynamics and persistence of CYP2D6 inhibition by paroxetine. J Clin Pharm Ther 2013; 38: 294–300.