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Evidence-based medicine for pharmacogenetics

Introduction

The variability of the response observed among patients to pharmacological treatments based on administration of standard doses of drugs is a well recognised problem in a physician's everyday practice. While the treatment can be largely ineffective in some individuals, the same dose can induce undesirable or even toxic effects in others. The response to treatment is generally affected by several environmental factors, such as co-medication, co-morbidity, age, nutrition and also by individual's genetics. The principal aim of pharmacogenetics is to determine the influence of the patient's genetic profile on the variability of the response to drugs in order to individualise and optimise the treatment. An important part of this genetic variability is due to common polymorphisms encountered in drug and xenobiotic metabolising enzymes, particularly within the superfamily of the hepatic P450 cytochromes (CYP). Although 58 different CYP genes have been described in humans, most of the currently administered drugs are the substrate of only a few members of this family. During the last three decades, many clinical studies have thoroughly investigated the role and consequence of the most common polymorphisms present in these enzymes (fig. 1) and recommendations for dose adjustments according to a patient's genotype have been proposed in several cases. However, in spite of the fact that pharmacogenetics is a field that has clearly entered an era of evidence-based medicine, both in terms of benefit for the patient and for the society (considering the cost of non-response to treatment and of drug-induced adverse effects from an

economical point of view), "pharmacogenotyping" has not yet been introduced into clinical practice in Switzerland, in part because this type of analysis is not reimbursed by social insurances. In this article, we review the evidence in the literature for the advantage of genotyping some CYP enzymes in particularly well characterised clinical situations.

CYP2D6

By itself, CYP2D6 is responsible for the metabolism of 20–25% of commonly administered drugs [1], including codeine, tramadol, tamoxifen, many antipsychotic, antiarrhythmic and antihypertensive drugs, most tricyclic antidepressants and some selective serotonin reuptake inhibitors (SSRI). This enzyme is probably the CYP isoform that has been the most extensively studied in pharmacogenetics and more than 100 alleles have been reported to influence its catalytic activity, with a regularly updated list (<http://www.cypalleles.ki.se/>). A poor metaboliser (PM) phenotype is observed in 5–10% of Caucasians and this is due to the presence in the same individual of two alleles without any activity (CYP2D6*3, *4, *5, or *6) [2]. The intermediate metaboliser (IM) pheno-

type, present in 10–15% of Caucasians, is mostly attributed to the reduced activity of allele *41. In Asian populations, this phenotype is more frequent and due to the presence of allele *10. In contrast the ultra-rapid metaboliser (UM) phenotype is due to gene duplication or multi-duplication. Interestingly, a North to South gradient (1 to 10%) has been revealed in European populations, with a higher prevalence of UM in southern Europe, possibly due to a selection pressure resulting from the presence of many CYP2D6 substrate alkaloids in southern European diet. Extensive metabolisers (EM), with normal CYP2D6 metabolic activity, account for about 65–80% of Caucasians.

Codeine and tramadol are central analgesics that must be activated by CYP2D6 to exert an opioid activity. Whilst CYP2D6 PMs have little benefit from these treatments, UMs are at risk of experiencing unwanted toxic effects. After the death of a newborn baby due to codeine intoxication through the maternal milk in 2007, the American Food and Drug Administration (FDA) has issued recommendations for the use of this drug by breast feeding mothers [3]. Tamoxifen, an adjuvant drug in breast cancer treatment, is also

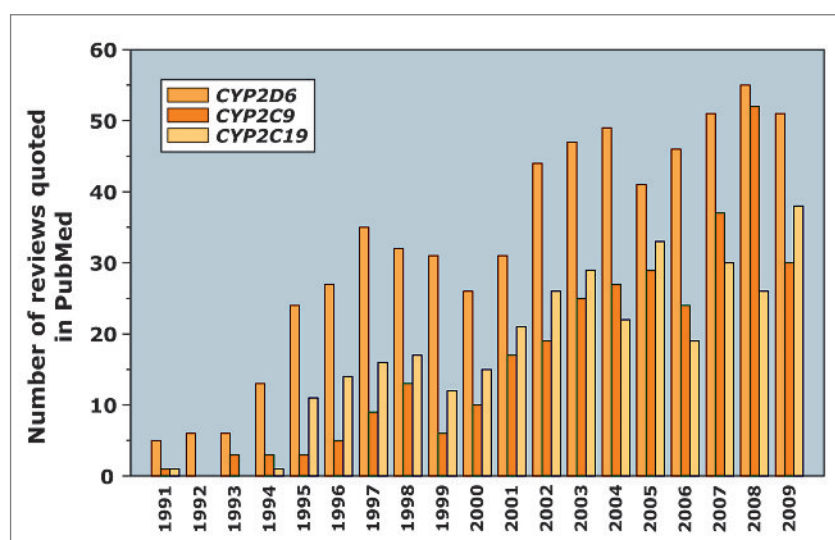


Figure 1

Shown are the number of reviews quoted each year since 1991 in PubMed found by searching with the key words CYP2D6, CYP2C9 and CYP2C19.

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a pro-drug activated by CYP2D6. Several early clinical studies suggested that the treatment is less efficient in CYP2D6 PMs and IMs, with a higher incidence of relapse in these patients, a finding recently confirmed in a large cohort of 1325 patients [4]. As alternative treatments with aromatase inhibitors are now available, genotyping CYP2D6 before initiating a treatment with tamoxifen seems indicated.

Dose adjustment recommendations as a function of CYP2D6 genotype have been proposed for all tricyclic antidepressants, for SSRIs (e.g., paroxetine) or selective serotonin and noradrenalin reuptake inhibitors (e.g., duloxetine and venlafaxine) [5]. Changing to another drug during antidepressant treatment has been shown to be more frequent in CYP2D6 PM than in EMs, and PM patients are over-represented in the group with adverse effects [6, 7]. The impact of the CYP2D6 genotype on the cost related to severe psychiatric diseases has been investigated in the US [8]. This study demonstrated that the number of adverse effects increases from UM to EM to IM and then to PM patients, and that the costs are higher in both UM and PM, while the hospitalisation duration is the highest in PM.

CYP2C9 and VKORC1

Treatment with anti-vitamin K (AVK) anticoagulants is very common and indicated in several pathological situations such as venous thrombosis, cardiac fibrillation, pulmonary embolism or cardiac valve replacement. Warfarin, or, more commonly used in Europe, acenocoumarol or phenprocoumon, are AVK anticoagulants principally metabolised by CYP2C9 [9]. They exert their anticoagulant activity by inhibiting the subunit 1 of the vitamin K epoxide reductase complex (VKORC1), thus preventing the regeneration of the reduced form of Vit K, required for the activation (carboxylation) of some coagulation factors. These drugs have a narrow therapeutic window and a large intra- and inter-individual variability of the drug response has been observed. The efficient dose necessary for reaching the therapeutic target varies from 1 to 10 among patients, depending on genetic factors amongst other factors

such as age, gender, body surface, hepatic function, Vit K uptake, alcohol consumption and co-medications [10, 11]. The safety of the treatment is guaranteed by monitoring its effects on coagulation through regular INR (International Normalised Ratio) determination, in order to prevent the deleterious consequences of either an overdose (haemorrhages) or a sub dosage (thrombo-embolic events). In order to optimise the choice of the initial dose, the role of influencing parameters, particularly genetic factors, has been extensively investigated.

Both CYP2C9 and VKORC1 have been clearly involved in the variability of individual responses to AVK drugs. The CYP2C9 polymorphism, by influencing the metabolism of these drugs, significantly affects the response to treatment. The presence of alleles *2 and *3, corresponding to a reduced activity of the CYP2C9 enzyme, is associated with an increased sensitivity to AVK drugs [12]. Heterozygotes in the Caucasian population for these two alleles are 22% and 15%, respectively, and they are at risk of experiencing haemorrhagic complications, particularly when initiating their treatment [13, 14].

Since the identification of the VKORC1 gene in 2004, several variants have been described and their association with variable response to AVK characterised [15, 16]. In addition to a few rare mutations found in patients resistant to AVK, two frequent polymorphisms in linkage disequilibrium, located in the first intron (c.1173C>T, rs9934438) and the gene promoter (g.-1639G>A, rs9923231), have been associated with a change of the sensitivity to AVK [17, 18], requiring an adjustment of the doses of warfarin or acenocoumarol. Important interethnic differences have been observed in the frequency of these alleles, differences that are correlated with the lower doses of warfarin generally required in Asian patients as compared to Caucasians for reaching the same levels of anticoagulation.

The influence of CYP2C9 and VKORC1 polymorphisms on pharmacokinetics and pharmacodynamics of AVK is now well established and their respective contribution to the inter-individual variability of the response is estimated

to be of 12% for CYP2C9 and of 30% for VKORC1 (while 17–22% is attributed to nongenetic factors) [19]. The FDA now requires the label of warfarin to mention that the optimal dosage can vary according to the patient's CYP2C9 and VKORC1 genotype and several algorithms have been developed for calculating initial doses, taking into account these genetic factors as well as other nongenetic factors, such as diet, gender or co-medication [20].

CYP2C19

Clopidogrel is widely used in patients after percutaneous coronary intervention and as a preventive treatment to avoid heart attack and stroke. Clopidogrel needs to be bio-activated by CYP3A4, CYP3A5 and CYP2C19. The CYP2C19 gene is polymorphic and has a number of loss-of-function (LOF) alleles. Carriers of CYP2C19 LOF alleles have a greatly diminished therapeutic response to clopidogrel (12% versus 8% in non-carriers) [21]. In CYP2C19 LOF carriers, the AUC of the clopidogrel active metabolite is <80% as compared to patients with the EM phenotype which reduces the therapeutic (cardiovascular) effect of clopidogrel [22]. Among the 1535 patients who underwent percutaneous coronary intervention during hospitalisation in this study, the rate of cardiovascular events in patients with two CYP2C19 LOF alleles was 3.58 times higher as compared to patients not carrying LOF alleles. The PM phenotype occurs in <7% of the Caucasian population. The most frequent allele with LOF is a single 681G>A substitution in exon 5 (CYP2C19*2), which creates a novel aberrantly spliced CYP2C19 mRNA which accounts for 75% of CYP2C19 null alleles in Caucasians. The second most common CYP2C19 allele (CYP2C19*3) associated with the PM phenotype results from a single nucleotide substitution G636A, which produces a premature stop codon and a failure to produce an active enzyme.

Voriconazole is widely used for the treatment of invasive fungal diseases. It is primarily metabolised by CYP2C19, CYP2C9 and CYP3A4. Polymorphisms within the CYP2C19 gene account for a relatively large proportion of the vari-

ability of voriconazole plasma concentrations. Four to five times higher voriconazole concentrations were measured in patients with a PM phenotype as compared to EM [23]. No significant relationship was found between CYP2C19 genetic polymorphisms, voriconazole trough concentrations and liver abnormalities in a Japanese population [24]. However, patients with the wild type genotype received different voriconazole dosages as compared to those with the mutant CYP2C19 genotype. Thus, the risk of hepatotoxicity increases with voriconazole plasma concentration. Most patients (75%) who developed hepatotoxicity showed voriconazole trough concentrations higher than 4 µg/ml. Thus, voriconazole daily doses corresponding to a trough concentration of 4 µg/ml were 8.9 mg/kg/day for patients with the wild type genotype and 6.5 mg/kg/day for the non-wild type genotypes.

Conclusion

In many instances, genotyping of drug metabolising enzymes or other phar-

macodynamic factors would allow the rapid identification and exclusion of patients at risk of experiencing adverse drug effects and those who cannot respond to the treatment. This information would help to individualise and optimise the treatment by selecting the most appropriate drug and/or by adapting the dose. Such genotyping would not replace but advantageously complement other clinical determinations, such as therapeutic drug monitoring of the plasma concentrations or functional tests (e.g., INR determinations). Since the genotype remains unchanged throughout life, only a single determination is required. Moreover, the information will be useful for any future treatment with drugs metabolised by the same pathway or drugs having the same mechanism of action. The different molecular biology methods used for genotype determination (in either a blood or saliva sample) are well standardised and commonly used in most laboratories. "Pharmacogenotyping" therefore appears to be an efficient test, not only



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for increasing therapeutic response and decreasing adverse effects, but also for preventing additional costs often associated with particular genotypes.

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