6. DOSE ADJUSTMENTS BASED ON PHARMACOGENETICS OF CYP450 ENZYMES

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6.1 Introduction

In today's medicine, drug therapy represents an important tool to treat or control disease. Dosing drugs is based on the assumption that a particular dose will yield a predefined concentration in the circulation and thereby establish the desired therapeutic effect. The fact that liver and kidney function may affect this relationship is appreciated, as is the fact that the use of co-medication potentially may interfere with the relationship between dose and blood concentration. Also environmental factors, like diet, alcohol use, smoking behaviour and caffeine intake may alter the rate at which particular drugs are metabolized, and will thus affect exposure of individuals to these drugs. A factor that has been neglected for a long time is genetics: differences in genetic make up may affect the relation between drug dose and blood concentration. Variant alleles encoding drug transporters or drug metabolizing enzymes with altered activity, present in the population, will give rise to unexpectedly high or low blood concentrations in response to a particular dose of a drug in certain individuals. The variation in drug metabolizing capacity between individuals is known for quite some time, but only recently this is becoming available as a routine diagnostic to provide better therapy. An important group of enzymes involved in drug metabolism is the cytochrome P450 enzyme system, which is mainly (but not only) active in the liver and the intestine. It consists of a family of isoenzymes, each with their own substrate specificity, and catalyzes oxidative reactions in order make potentially toxic and harmful substances more soluble. Purpose is to protect the body against toxic substances. The most important cytochromes with respect to drug metabolism are CYP3A4, CYP2C9, CYP2C19 and CYP2D6. For these enzymes, however, several variant alleles encoding enzymes with decreased activity exist (see www.cypalleles.ki.se), giving rise to interindividual differences in capacity to metabolize drugs. The best known example in this family is the enzyme CYP2D6, for which even 10% of the Caucasian population is a poor metabolizer, due to inheritance of two inactive variant alleles.

The documented genetic variability in cytochrome P450 enzymes has increased considerably the last years, and it has become clear that inherited capabilities of drug metabolism by this important detoxifying system may be of importance for predicting outcome of drug treatment. Especially the fact that this concerns inherited information makes it possible to assess prospectively the drug metabolizing capacity of patients. Whereas detection of variant alleles is an important first step, the real clinical benefit should come from adjusting drug therapy based on this genetic information. It is at this stage where the field of pharmacogenetics nowadays finds itself.

6.2 CYP450 isoenzymes and variant alleles

6.2.1 CYP2D6

CYP2D6 is the best characterized polymorphic cytochrome P450 enzyme to date. Whereas it represents only 2% of total CYP450 protein in the liver, it is involved in the metabolism of approximately 20-25% of commonly prescribed drugs. Based on debrisoquine and sparteine phenotyping, the Caucasian population was shown to be divided in poor (5-10%), extensive (90%) and ultra extensive (or ultra rapid) (1-2%) metabolizers. This trimodal distribution proved to be caused by genetic factors. Already in 1977, the most common CYP2D6 null (deficient) allele, CYP2D6*4 (1846G>A, splicing defect), was discovered. This variant has an allelic frequency of 20%, and is thus not a rare allele. Also a CYP2D6 gene deletion (CYP2D6*5 variant allele) is not uncommon: it has an allele frequency of 3.8%. Together with the simultaneous analysis for CYP2D6*3 (2549delA, frameshift; allele frequency 1-2%) and CYP2D6*6 (1707delT, frameshift; allele frequency 1%), over 98% of CYP2D6 poor metabolizers can be identified in the Caucasian population with these tests. At this moment, many more variant alleles for CYP2D6 have been described (up to CYP2D6*63 at this moment (www.cypalleles.ki.se)). These variant alleles can be divided in functional alleles (like *1, *2, *35, encoding normal activity), null alleles (like *3, *4, *5, encoding no activity at all) and decreased activity alleles (like *9, *10, *41), encoding enzymes with residual metabolic capacity. Based on genotyping, we can predict CYP2D6 extensive metabolizers (EMs; normal metabolism: 2 active alleles)), intermediate metabolizers (IMs; only 1 active allele), poor metabolizers (PMs; 2 inactive alleles) and ultra rapid metabolizers (URs or UEMs; gene duplication). However, the overlap in enzyme activity between these groups can be substantial, and also depends on the drug. In addition, CYP2D6 variant alleles show a large difference in distribution between ethnicities: the CYP2D6*10 decreased activity allele is present in a large proportion of Asians, and causes a shift towards a lower mean CYP2D6 activity for this population compared to Caucasians. The CYP2D6 gene duplication occurs in approximately 1-2% of Swedes, 3.6% of Germans, 7-10% of Spaniards, 10% of Italians, 20% of Saudi Arabians and 29% of black Ethiopians, thus revealing a strong north-south gradient, probably caused by evolutionary pressure with respect to diet. Based on the occurrence of alleles, a semi-quantitative gene dosage (SGD) score was introduced by Steimer *et al*, to better predict CYP2D6 activity. In this scoring system, active alleles were assigned a score 1, decreased activity alleles a score 0.5 and null alleles a score 0. This leads to SGD groups of 0, 0.5, 1, 1.5, 2 and >2.

6.2.2 The CYP2C subfamily

The CYP2C subfamily consists of four genes, located on chromosome 10q24. CYP2C9 is the main enzyme of the CYP2C subfamily in the human liver, and is involved in the metabolism of 10-20% of commonly prescribed drugs. Approximately 1-2% of the Caucasian population is a poor metabolizer for CYP2C9, while for Africans and Asians this percentage is thought to be much lower. The CYP2C9*2 (430C>T, Arg144Cys) and CYP2C9*3 (1075A>C, Ile359Leu) variant alleles encode both significant altered CYP2C9 activities and do show high population frequencies: 11% (CYP2C9*2) and 7% (CYP2C9*3) in Caucasians, 4% (CYP2C9*2) and 2% (CYP2C9*3) in Africans and 0% (CYP2C9*2) and 3% (CYP2C9*3) in Asians. The decrease in activity is most profound for the CYP2C9*3 allele: mean clearances in homozygote CYP2C9*3/*3 individuals are 25% of that of wild type for a number of substrates, while heterozygosity for this variant corresponded to clearance of 29% compared to wild type. For the CYP2C9*2 allele, the Vmax displays a 50% reduction compared to the CYP2C9*1 allele and residual clearance of tolbutamide for CYP2C9*1/*2 heterozygotes was 70% compared to

CYP2C9*1/*1 individuals. In total, approximately 30 CYP2C9 variant alleles have now been described.

The second important enzyme of the CYP2C subfamily is CYP2C19, for which the first genetic polymorphism was identified based on aberrant metabolism of the anticonvulsant drug mephenytoin: 3-5% of Caucasians and 12-23% of Asians were shown to be CYP2C19 poor metabolizers. In African populations, 4% CYP2C19 poor metabolizers can be found. The two predominant variant alleles, encoding CYP2C19 protein lacking enzymatic activity, are CYP2C19*2 (681G>A, splicing defect) and CYP2C19*3 (636G>A, W212X). At present, nomenclature has reached the CYP2C19*21 allele, and does include several null alleles with relative low allelic frequencies. An interesting allele, CYP2C19*17 (991A>G, Ile331Val), was recently characterized as encoding an increased activity on proton pump inhibitor metabolism, due to increased transcription compared to the CYP2C19*1 allele, predicting 35-40% higher activity in homozygote individuals. Also part of the CYP2C family is the CYP2C8 enzyme, which plays a role in paclitaxel metabolism. CYP2C8 has variant alleles CYP2C8*2, *3, *5, *7 and *8 allele that were shown to have decreased activity. From these alleles, the CYP2C8*2 allele is found predominantly in African Americans (allele frequency of 18%) and has a low frequency in Caucasians (allele frequency 0-0.7%). In contrast, the CYP2C8*3 allele was found in 2% of African Americans and in 8-13% of Caucasians.

6.2.3   The CYP3A subfamily

The CYP3A subfamily gene cluster is located on chromosome 7; this family is responsible for the metabolism of approximately 50% of all drugs. It is the largest subfamily of CYP450 enzymes in the liver, and comprises the isoenzymes CYP3A4, CYP3A5, CYP3A7 and CYP3A43. For CYP3A4, the most important member of this subfamily, over 20 genetic polymorphisms have been described, but almost all have low (<1%) allelic frequencies making them unlikely targets for pharmacogenetic screening. The only exception on this might be the promoter variant CYP3A4*1B (-392A>G), originally referred to as CYP3A4-V, which has an allelic frequency of 2-9% in Caucasians, 35-67% in African Americans and which is rare in Asians. This polymorphism is associated with a moderate (1.2-1.9 fold) increase in transcription rate and thus enzymatic activity, although this effect was questioned by other authors. Because CYP3A expression is highly susceptible to induction and inhibition of transcription, it is not clear whether this modest difference in induction of the CYP3A4*1B allele will contribute significantly to the observed 40-fold variation in CYP3A4 activity in vivo. From the other genetic polymorphisms, the CYP3A4*2 (664T>C, Ser222Pro) and CYP3A4*17 (566T>C, Phe189Ser) were shown to have decreased enzymatic activity, while this is also suggestive for CYP3A4*4 (352A>G, Ile118Val) and CYP3A4*5 (653C>G, Pro218Arg). In addition, two null alleles have been identified: CYP3A4*6 (830-831insA; frameshift) and CYP3A4*20 (1461-1462insA; frameshift), but these alleles are extremely rare. In addition, the CYP3A4*18 (878T>C, Leu293Pro) variant allele surprisingly was shown to encode increased enzymatic activity. This allele is not found in Caucasians, but has an allele frequency of 10% in Chinese.

A second member of the CYP3A family, CYP3A5, is not expressed an all Caucasians. In 10-40% of this population, homozygosity for the inactive CYP3A5*3 allele causes absence of protein and thus CYP3A5 activity. The allele frequency of the CYP3A5*3 allele varies from 89-94% in Caucasians, 71-75% in East Asians, 60-65% in Hispanics to 29-35% in Blacks. At this moment, 13 genetic polymorphisms have been described fro CYP3A5, but CYP3A5*3 seems to be by far the major variant allele. The relevance of CYP3A5 genotyping will depend

on the contribution of this enzyme to total CYP3A mediated metabolism of a specific drug. For many drugs, this contribution is not (yet) exactly known.

The CYP3A cluster contains also two minor CYP3A genes: CYP3A7 and CYP3A43. CYP3A7 is expressed mainly during fetal life and is down regulated after birth. However, 3% of Caucasians and 6% of African Americans still express CYP3A7 during adult life due to inheritance of the CYP3A7*1C allele, which contains 60 bp of the CYP3A4 promoter. Up to now, no correlations with altered pharmacokinetics with specific drugs have been described, but a correlation with blood levels of the CYP3A7 substrate DHEAS apparently exists, demonstrating an impact of this polymorphisms in adults. The role of CYP3A43 with respect to drug metabolism is unclear at the moment This enzyme is expressed in very low levels in human liver, and no information is available as to which drug metabolism it might contribute.

6.3   Dose adjustments based upon CYP450 genetic polymorphisms

6.3.1   Pain

CYP2D6 plays an important role in the metabolism of codeine, used to treat pain. Codeine needs to be activated by conversion to morphine by CYP2D6 in order to achieve pain relieve. Because of this activation, CYP2D6 poor metabolizers will not benefit from codeine as pain medication. Dose adjustments are thus not appropriate, and another analgesic should be considered. Knowledge about drug metabolism is important here, because also the analgesic tramadol requires activation by CYP2D6. Another important problem may arise for CYP2D6 ultra rapid metabolizers: because of the increased conversion of codeine to morphine, severe morphine toxicity may occur. Several case reports have been published on this, with a tragic story in 2006 in the Lancet of a new born child dying on day 13 because his mother was on codeine. Because the mother was a CYP2D6 ultra rapid metabolizer, the normal codeine dose translated in fatal morphine concentrations in the mothermilk. Whereas dose adjustments could be appropriate here, it is much easier to prescribe another analgesic lacking these dangerous side effects.

6.3.2   Psychiatry

Important, and widely used substrates of CYP2D6 are antidepressants (tricyclic antidepressants and SSRIs) and antipsychotics. CYP2D6 genotype was shown to correlate strongly with drug metabolism. Based on available literature, Kirchheiner *et al* extrapolated dose recommendations based on genotype for several antidepressants and antipsychotics. From this work, it is very clear that although multiple drugs are a substrate of CYP2D6, the clinical consequence of being a poor metabolizer very much depends on the drug. As example: whereas CYP2D6 poor metabolizers would need a 72% reduction in dose compared to standard dosing to reach target concentrations, citalopram needs to be adjusted only 2%, whereas both drugs are CYP2D6 substrates. In our own hospital, we showed that the obtained steady state dosages for imipramine could be well predicted by CYP2D6 semi quantitative gene dosage, and indeed got a 70% dose reduction for imipramine in CYP2D6 PMs. For IMs, slightly higher doses were found than predicted by Kirchheiner *et al*, indicating that clinical validation of extrapolated data is important. Also apparent was that considerable overlap exists between different genotyping groups. Pharmacogenetic based dosing is thus certainly possible, but it cannot replace therapeutic drug monitoring.

6.3.3   Anticoagulation

A major topic in today's medicine is the use of CYP2C9 genotyping to improve anticoagulant therapy: warfarin and acenocoumarol are both depending on CYP2C9 activity for metabolism. Based on studies on warfarin, special websites (like www.warfarindosing.com) with algorithms are now available which will enable physicians to target the INR of patients more quickly, based on CYP2C9 genotyping information. In addition, the genetic information on the polymorphic VKORC1 gene, encoding the target molecule for warfarin and acenocoumarol, needs to be provided to be able to retrieve the optimal dosing. This system seems to be now the most advanced with respect to CYP450 genetic information in relation to dose prescription.

6.3.4   Immunosuppressants

One of the first drugs in which CYP3A5 proved of importance, is the immunosuppressant tacrolimus, used in kidney transplantation. CYP3A5 expressers (20% of Caucasians) require 1.5 – 2.0 fold higher tacrolimus dosages to obtain target concentrations. Although there is a difference in drug metabolism rate between CYP3A5 expressers and non-expressers, this did not translate into differences in kidney transplantation rejection, making the clinical utility yet unproven. Currently, a prospective trial is performed in which tacrolimus dosing is based on CYP3A5 genotype, which should demonstrate the clinical relevance of CYP3A5 genotyping.

6.3.5   Cancer

The most challenging field for applying molecular genetics to predict drug response, is cancer. Also here, CYP2D6 has become important the last years, since it catalyzes the activation of tamoxifen, which is used in the treatment of breast cancer. CYP2D6 PMs do benefit less well from standard tamoxifen therapy. Therefore, the FDA is considering to indicate CYP2D6 genotyping in the label for tamoxifen. However, it is currently not clear whether increased tamoxifen dosages should be used, or that alternative drugs (like aromatase inhibitors) should be considered. Other interesting findings in this field are correlations of CYP2C8 genotype with paclitaxel metabolism, and CYP2B6, CYP2C19, CYP3A4 and CYP3A5 genotype with cyclophosphamide and ifosfamide effectivity. Also etoposide and vincristine metabolism were correlated with CYP3A5 genotype, and several studies have investigated the role of CYP450s in the metabolism of IRESSA, Gleevec, irinotecan and docetaxel. Most of these studies, however, do not yield consistent results to favour CYP450 genotyping.

Recommended literature:

1.   Murray M, Petrovic N. Cytochromes P450: decision-making tools for personalized therapeutics. Curr Opin Mol Ther 2006;8(6):480-6.
2.   Kirchheiner J, Nickchen K, Bauer M, Wong ML, Licinio J, Roots I, *et al*. Pharmacogenetics of antidepressants and antipsychotics: the contribution of allelic variations to the phenotype of drug response. Mol Psychiatry 2004;9(5):442-73.
3.   Schenk PW, van Fessem MA, Verploegh-Van Rij S, Mathot RA, van Gelder T, Vulto AG, *et al*. Association of graded allele-specific changes in CYP2D6 function with imipramine dose requirement in a large group of depressed patients. Mol Psychiatry 2007.
4.   Van Schaik RH. Cancer treatment and pharmacogenetics of cytochrome P450 enzymes. Invest New Drugs 2005;23(6):513-22.

5.   Zhu Y, Shennan M, Reynolds KK, Johnson NA, Herrnberger MR, Valdes R Jr, *et al.* Estimation of warfarin maintenance dose based on VKORC1 (-1639 G>A) and CYP2C9 genotypes. Clin Chem. 2007;53(7):1199-205.

6.   Steimer W, Zopf K, von Amelunxen S, Pfeiffer H, Bachofer J, Popp J, *et al.* Allele-specific change of concentration and functional gene dose for the prediction of steady-state serum concentrations of amitriptyline and nortriptyline in CYP2C19 and CYP2D6 extensive and intermediate metabolizers. Clin Chem. 2004;50(9):1623-33.