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## Pharmacogenetics: from research to clinical implementation

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In today's medicine, patients are receiving drug therapy using dosages which have been established as the best dose, determined as an average in a large group of individuals. Although we take for granted that patients behave in a similar way on drug therapy, it is well known from clinical practice that interindividual variations exists in drug response. For drugs with a narrow therapeutic window, and/or drugs with potentially severe or even lethal side effects, this constitutes a major problem. Adverse Drug Reactions (ADRs) affect 2 million patients/year in the USA, resulting in 100,000 deaths annually. This makes ADRs the 5th most frequent cause of death. In fact, 7% of all hospitalizations are caused by ADRs (1-4). On the other side, of all drugs, only 25-60% are effective. A major part of the variability in drug response is thought to be the consequence of substantial interindividual variability in drug metabolism. This metabolism of drugs

by the liver is partly determined by hereditary factors, with variant alleles of the same gene potentially encoding active, inactive or ultra-active enzyme activities. Using pharmacogenetics, being DNA analyses in genes encoding drug metabolizing enzymes and drug transporters, the challenge is to identify patients with these genetic variants, thereby predicting their corresponding metabolic capacity. With this information, genotype adjusted dosages, or another drug can be prescribed. This Personalized Medicine approach can improve healthcare by decreasing ADRs and improving effectivity of treatment, a topic of interest for patients and healthcare professionals with, in addition, substantial economical implications as well. The current challenge is therefore to characterize to what degree these genetic polymorphisms affect drug therapy. This would enable the identification of those pharmacogenetic markers that could help in routine clinical practice to explain, or preferably predict aberrant reactions to common drug therapies.

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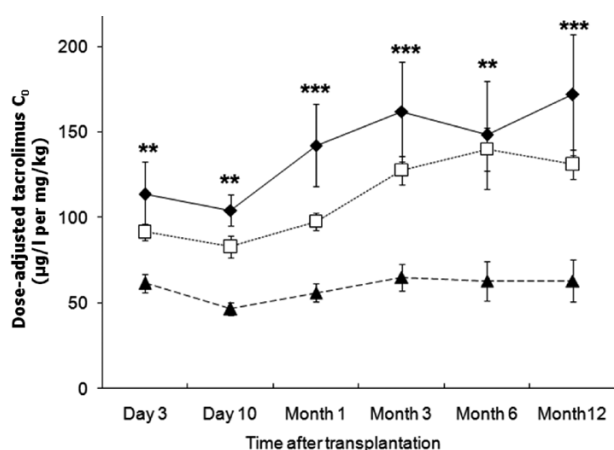
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### Immunosuppressants: tacrolimus, cyclosporine and MMF

The most prevalent treatment in immune suppression for solid organ transplantation is a combination of mycophenolate mofetil (MMF), a calcineurin inhibitor (tacrolimus or cyclosporine) and glucocorticoids. Tacrolimus has a narrow therapeutic window. Underexposure may lead to acute graft rejection whereas overexposure can result in side effects, of which nephrotoxicity is the most troublesome. For

this reason, therapeutic drug monitoring (TDM) is essential to guide therapy. Tacrolimus displays a high interindividual variation in pharmacokinetics, which raises the question to what extent genetic determinants are responsible for this. The metabolism of tacrolimus is mainly dependent on the cytochrome P450 3A (CYP3A) system, predominantly CYP3A4. For the gene encoding this enzyme, however, no clinically important (based on effect on enzyme activity and allele frequency) polymorphisms have been described thus far (5). For CYP3A5, approximately 80% of the Caucasian population has two inactive *CYP3A5*\*3 alleles (6), classifying them as CYP3A5 non-expressers. In 2003, we demonstrated as one of the first groups worldwide that the pharmacokinetics of tacrolimus was heavily dependent on *CYP3A5* allelic status (7). CYP3A5-expressers, being patients with the *CYP3A5*\*1/\*1 or *CYP3A5*\*1/\*3 genotype, needed twice the amount of tacrolimus as compared to CYP3A5 non-expressers to reach comparable predose concentrations (7), a finding which has now been confirmed by many others. We could also demonstrate that this effect of *CYP3A5* genotype on tacrolimus PK was present in children (8). A recent randomized controlled clinical trial was published that demonstrated that CYP3A5 genotype guided tacrolimus dosing in kidney transplant patients led to a more rapid achievement of the tacrolimus concentration within the therapeutic window and significantly less dose adjustments (8). In 2011, a new intronic SNP in *CYP3A4* was described that affected CYP3A4 activity. In our MMF Fixed-Dose versus Concentration-Controlled (FDCC)-study, we showed that this new *CYP3A4* SNP contributes independently of *CYP3A5* genotype to tacrolimus metabolism, with a comparable effect size as the *CYP3A5* SNP (9-10). Dividing patients in CYP3A4 combined *CYP3A4*/*CYP3A5* genotype groups, this provided significant predictions of tacrolimus pharmacokinetics (figure 1). For cyclosporine, another immunosuppressant with a



**Figure 1.** Effect of *CYP3A4*/*CYP3A5* combined genotype groups on tacrolimus dose-adjusted predose concentrations. Poor Metabolizers: *CYP3A5* non-expressers + *CYP3A4*\*1/\*22 patients, Intermediate Metabolizers: *CYP3A5* non-expressers + *CYP3A4*\*1/\*1, Extensive metabolizers: *CYP3A5* expressers + *CYP3A4*\*1/\*1. *CYP3A5* expressers: *CYP3A5*\*1/\*1 + *CYP3A5*\*1/\*3 patients. —◆— Poor metabolizers (group 1); ---□--- Intermediate metabolizers (groups 2 + 3); -▲- Extensive metabolizers (group 4).

narrow therapeutic window and a large interindividual variation in pharmacokinetics, the contribution of genetic polymorphisms was less clear. CYP3A5 does not seem to play a major role and only a minor contribution of the *CYP3A4*\*1*B* promoter polymorphism could be demonstrated, using population pharmacokinetics (7, 10-12). The other component of the immunosuppressive regimen, MMF, this drug is hydrolyzed by esterases to its active metabolite mycophenolic acid (MPA). MPA is inactivated by glucuronidation through UDP-glucuronosyltransferase 1A9 (*UGT1A9*) into MPA-glucuronide. MPA plasma concentrations on day 3 after transplantation were correlated with risk of biopsy proven acute rejection (BPAR) in the first month ( $p=0.009$ ) and first year ( $p=0.006$ ) (13) after transplantation, indicating that prediction of MPA metabolism might be of clinical importance. Thus far, the issue of TDM for MPA is still under debate. The absence of TDM, however, could increase the potential contribution of a pharmacogenetic marker. Using the large cohort of the FDCC-study, we demonstrated that in patients on a tacrolimus/MMF regimen, the increased-activity *UGT1A9* -275T>A genetic polymorphism correlated significantly with a 20% lower MPA exposure, and with a significantly higher risk on biopsy proven acute rejection (OR 13.3;  $p<0.05$ ) (14). Interestingly, this effect of the *UGT1A9* promoter polymorphism was absent in the MMF/cyclosporine treated patients.

In summary, for immunosuppression therapy, genetic polymorphisms in *CYP3A4*, *CYP3A5* and *UGT1A9* have a significant influence on the pharmacokinetics of tacrolimus and MMF, respectively. At present, pretransplant genotyping for CYP3A5 in patients that will receive tacrolimus appears to be the most promising application of pharmacogenetics in this field. To further evaluate its use in routine practice, a prospective study in kidney transplant patients is currently in progress, investigating the potential benefit of tacrolimus tailored dosing, based on the recipients *CYP3A5* genotype (NTR 2226, [www.trialregister.nl](http://www.trialregister.nl); Hesselink et al., personal communication).

### Oncology: tamoxifen and taxanes

The best described and well known polymorphic enzyme of the CYP450 family is CYP2D6. Being involved in the metabolism of 25% of drugs, with a frequency of 5-10% poor metabolizers in the Caucasian population, genetic polymorphisms in *CYP2D6* may have profound effects on drug therapy. Thus far, over 80 variant alleles have been described in the population, but most of them with a relatively low frequency. The most abundant polymorphic allele in the Caucasian population is the *CYP2D6*\*4 allele (minor allele frequency 18-22%), which is characterized by the 1846G>A polymorphism, affecting a splice site in the gene. Generally, *CYP2D6* alleles can be divided into active alleles (\*1, \*2, \*35), decreased activity alleles (like \*9, \*10, \*17, \*29, \*41) and inactive or null-alleles (like \*3, \*4, \*5, \*6, \*7, \*8, \*11, \*14, \*15, \*19, \*20, \*40). A drug that is dependent on an activation step by CYP2D6 is the anti-estrogen tamoxifen, which is

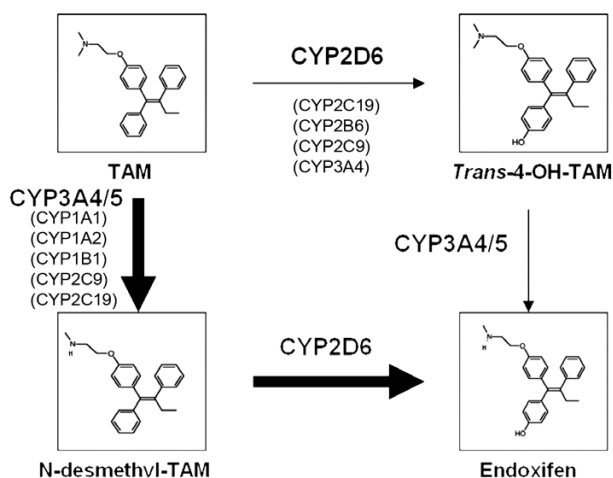
used in the treatment of breast cancer. The conversion of tamoxifen into the active component endoxifen is almost completely dependent on CYP2D6 activity (figure 2). Studies showing a decreased concentration of endoxifen in CYP2D6 poor metabolizers have been published, and a lower breast cancer survival for CYP2D6 poor metabolizers in adjuvant tamoxifen treatment has been described by several groups. We also confirmed this effect in breast cancer patient receiving tamoxifen in the Rotterdam study (15-16). Although we did find a worse treatment outcome based on CYP2D6 genotype status in patients treated with tamoxifen for metastatic disease instead of as adjuvant treatment (15-16), we were not able to confirm this in three other independent cohorts of a multicenter study of 499 patients (17). This controversy seems to be a major problem into today's discussion about whether or not to implement *CYP2D6* genotyping for patients that will receive tamoxifen. Yet, the importance of CYP2D6 activity was also demonstrated by the fact that antidepressant use, especially with a strong inhibitory effect on CYP2D6, negatively affected outcome on tamoxifen adjuvant therapy. The possibility to use phenotyping instead of genotyping, in order to detect both genotype and CYP2D6 inhibitory co-medication, was investigated by using dextromethorphan as a probe drug for endoxifen exposure, with promising results (18). Recently, we identified quite unexpected another genetic marker for tamoxifen therapy: the *CYP2C19*\*2 allele. This variant allele predicted a better response to tamoxifen in metastatic breast cancer patients. This effect was demonstrated in four independent cohorts (17, 19) but thus far the underlying mechanism remains unresolved, and is subject of current and future studies. The issue of whether or not to determine CYP2D6 genotype status prior to starting tamoxifen therapy is currently a hot topic. Although evidence of the lower endoxifen levels in CYP2D6 variant allele carriers is undisputed, there are several studies showing a significant decreased disease free survival for CYP2D6

variant allele carriers but there are also several studies not showing this effect. The 'KNMP-Kennisbank' has included an advice to consider another drug than tamoxifen for CYP2D6 poor metabolizers but this advice is not part of the current guideline of the Dutch Oncologists on tamoxifen use as adjuvant therapy. Recent studies have shown that a decreased CYP2D6 metabolism can be at least partially overcome with respect to endoxifen levels by increasing the amount of tamoxifen from 20 to 40 mg. Several patients are specifically requesting *CYP2D6* genetic testing for their tamoxifen therapy, based on information available on the internet. It seems there is too much evidence to ignore the impact of CYP2D6 testing, yet not enough evidence to get in generally accepted. Time will tell how this important issue will further develop.

Docetaxel and paclitaxel are two taxane drugs used in the treatment of ovarian, oesophageal, prostate and breast cancer. Serious adverse effects are neutropenia and neutropenic fever for docetaxel and neurotoxicity for paclitaxel. Paclitaxel is metabolized mainly by CYP2C8, with a minor contribution for CYP3A4, whereas docetaxel is mainly depending on metabolism by CYP3A4. Both drugs are, however, also substrates for drug transporters, and genetic polymorphisms in these transporters could also influence the exposure to taxanes. We demonstrated a 64% increase in docetaxel clearance correlated with the *CYP3A54\*1B/CYP3A5\*1* combined genotype (20). Currently, we are investigating 700 paclitaxel and docetaxel treated patients in one of the largest studies worldwide on taxanes, using the Affymetrix Drug Metabolizing Enzyme and Transporter (DMET) chip that contains 1,936 SNPs in 223 drug metabolizing and transporter genes. Up to know, however, there is insufficient evidence for clinical implementation of any genetic marker for taxane therapy.

### Pain treatment and sedation

One of the most challenging fields to operate in for the identification of pharmacogenetic markers, is pain treatment. Common drugs like codeine, tramadol and oxycodon are substrates for the highly polymorphic CYP2D6 enzyme. Codeine and tramadol need activation by CYP2D6, thus causing undertreatment in CYP2D6 poor metabolizers. Several case reports or ADRs caused by CYP2D6 genetic status on tramadol and codeine have been published, yet thus far there seems to be no real incentive to consider routine genotyping prior to the treatment of pain. The FDA, however, did now include a black box warning concerning CYP2D6 in the drug label of codeine, indicating the potential severity of this gene-drug interaction. Although the expression of cytochrome P450 enzymes is still maturing in young patients, potentially obscuring any effects of genetic variation, we were able to identify the effect of CYP2D6 poor metabolizer status on tramadol O-demethylation pharmacokinetics in neonates and paediatric patients (21-22). For opioids like morphine or fentanyl, the dosage required to treat pain effectively in neonates is determined by experienced nurses using validated pain scores. We are currently



**Figure 2.** Metabolism of Tamoxifen (TAM). The formation of the most active metabolite endoxifen depends on the activity of the polymorphic enzyme CYP2D6. CYP2D6 Poor Metabolizers have significantly lower endoxifen concentrations and may therefore be at a higher risk of breast cancer recurrence in adjuvant tamoxifen users.

investigating which genetic polymorphisms could be used as an indicator for morphine requirement. In line with the involvement of UGT2B7 in the glucuronidation of morphine, we found that a polymorphism in the UGT2B7 promoter region (-840G>A) correlated with clearance of morphine in sickle cell patients (23). Preliminary results on neonates and young children identified COMT and  $\beta$ -arrestin2 as genes potentially affecting morphine requirement (van Schaik 2007, abstract IATDMCT, Nice; Norman 2012, abstract IASP, Miami) whereas also the influence of the A118G polymorphism in the OPRM1 gene, encoding the  $\mu$ -opioid receptor is worthwhile investigating. Regarding clinical implementation, there does not seem to be a major interest at present for implementing CYP2D6 genotyping for codeine, tramadol or oxycodon therapy, although exactly this has been suggested (and is under debate) for mothers breastfeeding while receiving codeine postpartum (24).

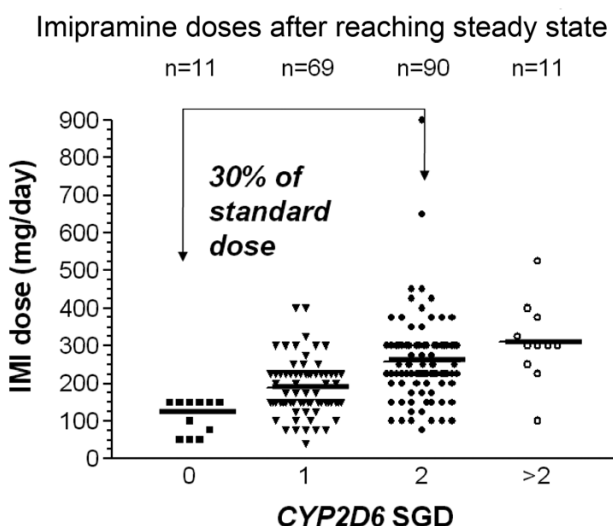
### Anticoagulation

One of the areas in which most adverse effects occur is that of oral anticoagulants. To guide coumarin therapy, frequent INR measurements are required. Yet, it is known that the coumarins warfarin, acenocoumarol (Sintrom) and phenprocoumon (Marcomar) are metabolized by CYP2C9, another polymorphic enzyme. In addition, coumarins achieve their effect by interacting with the Vitamin K Oxide Reductase complex (VKORC1), an enzyme involved in the formation of reduced vitamin K, which is needed to activate clotting factors. For VKORC1, genetic variants that yield a significantly higher sensitivity to coumarins have been identified: -1639G>A and 1173 C>T, being part of the same haplotype (25). Taken together, CYP2C9 and VKORC1 genotypes were able to predict 55% of war-

farin dose variation in the population (26). In response to this, the Food and Drug Administration (FDA) has included CYP2C9/VKORC1 genotype based dose recommendations in the warfarin drug label. In the Netherlands, mainly acenocoumarol and phenprocoumon are used as anticoagulants. We demonstrated by both a candidate gene approach as well as by a genome-wide association study that CYP2C9 and VKORC1 genotypes are the best predictors of acenocoumarol maintenance dose (27-30). The issue of whether or not we should genotype our patients treated with coumarin for CYP2C9 and VKORC1 is currently being debated. It seems that our well organized patient care regarding INR measurements reduces the potential contribution of a pharmacogenetic test, although a comparative trial in the US demonstrated a 25% reduction in hospitalization when genotyping was introduced. The 'KNMP-Kennisbank' also indicates that the CYP2C9/VKORC1 genotype affects acenocoumarol therapy (31).

### Psychiatry

The most promising field for application of CYP2D6 testing is Psychiatry. Many antidepressants and antipsychotics are metabolized by this polymorphic enzyme, and a correlation between CYP2D6 genotype and maintenance dose has been established (32). For the tricyclic antidepressant imipramine, we confirmed that imipramine maintenance dose was significantly dependent on CYP2D6 genotype: in a large cohort of 181 depressed patients, CYP2D6 poor metabolizers needed on average only 30% of the standard dose to reach therapeutic plasma concentrations, and a significant correlation with the number of active CYP2D6 alleles was seen (33). However, a substantial overlap for imipramine steady state dosage between the genotype groups was apparent (figure 3). In addition, the ultra-active CYP2C19\*17 allele had a significant effect role in the conversion of imipramine into desipramine (34), but as both imipramine and desipramine are active compounds, the clinical utility of genotyping for CYP2C19\*17 is limited. In clinical practise, the predictive power of CYP2D6 testing for antidepressants or antipsychotics is hardly used. We do see, however, requests for genotyping psychiatric patients that did experience side effects for which the psychiatrists wanted an explanation, also with respect to the choice of another drug. Also requests to distinguish between non-compliance and CYP2D6 ultra-rapid metabolism is frequently encountered in our patient diagnostic setting, indicating that on the diagnostic side, there seems to be a place for CYP2D6 genotyping in Psychiatry. The KNMP/WinAp group has formulated adjusted dosages for almost 50 drugs, including many antidepressants and antipsychotics (31).



**Figure 3.** The maintenance dose of imipramine (IMI) in depressed patients is significantly ( $p<0.05$ ) correlated with the number of active CYP2D6 alleles (SGD=Semi-quantitative Gene Dosage: 2 deficient alleles = SGD 0 (Poor Metabolizers), 1 active + 1 deficient allele = SGD 1 or two decreased activity alleles (Intermediate Metabolizers), 2 active alleles = SGD 2 (Extensive Metabolizers), gene duplication = SGD>2), but there is substantial overlap between genotype groups (adapted from (33)).

### Clinical implementation

The challenge of pharmacogenetic research is to get the applications implemented into clinical diagnostics. Besides solid evidence like randomized controlled trials and education, uptake in clinical guidelines is important. Since genetic polymorphisms in cytochrome P450 genes will have implications for several drugs, it is also of importance that pharma-

cists and physicians have access to information as to which drugs are affected and into what degree. The FDA keeps a database with 98 drugs that currently have pharmacogenetic information included in the drug label ([www.fda.gov/Drugs/ScienceResearch/ResearchAreas/Pharmacogenetics/ucm083378](http://www.fda.gov/Drugs/ScienceResearch/ResearchAreas/Pharmacogenetics/ucm083378)). For the Netherlands, the KNMP/WinAp has set up an expert group of pharmacists and clinical chemists to provide genotype based dose recommendations. This information is uniform for all pharmacies in the Netherlands, being broadly accessible through the KNMP-Kennisbank and which has also been published in the international peer-reviewed literature (31). Also a more strategic document addressing implementation of pharmacogenetic testing was recently published (35). Further harmonization in pharmacogenetics for routine patient diagnostics will be propagated by the recently initiated Dutch Clinical Pharmacogenetics Network that the objective to optimize our current knowledge and technical potential in order to broaden the application of true Personalized Medicine (CMBD Pharmacogenetics Symposium, Utrecht, Nov 2011).

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## Lab-on-a-chip technology for clinical diagnostics: the fertility chip

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In the 1990s the term micro total analysis systems ( $\mu$ TAS) was introduced to describe a complete micro-system which integrates sample handling, analysis and detection into a single device, also called Lab-on-a-Chip (LOC) device (1). The LOC concept defines the scaling down of a single or multiple lab processes into a chip format with dimensions as small as a stamp. Scaling down offers many advantages, such as less sample, reagent and waste volumes, faster analysis, integration of many analytical processes within one device, lower cost, to name a few, but first of all an easy handling. These advantages meet the actual demands of clinical laboratories, which are dealing with an increasing workload and decreased funding. Our group showed previously these advantages of the LOC technology for blood electrolyte determinations in clinical diagnostics (2).

Furthermore, microfluidic dimensions (10 - 100  $\mu$ m) equal the size of cells, making these devices very suit-

able for the analysis of many different biochemical processes even on a single-cell level. Hence, there are many reasons why microtechnology is advantageous compared to existing conventional analysis methods, especially in the case of cellular based assays, to understand how cells react in a certain environment, to a certain drug or in contact with other cell types. Different cell manipulation methods (e.g. sorting, detachment, staining, fixing, lysis) can be integrated on one chip, less sample is needed ideally when only a few cells are available (e.g., primary cells) and the dimensions favour single-cell analysis. Furthermore, optical detection techniques can be automated and in some cases be replaced by electrical on-chip detection methods. Moreover, development of cell arrays, which are analogous to DNA or protein arrays, offer the possibility for high-throughput screening. Recent technological developments enable detailed cellular studies, defining a new concept: Lab-in-a-Cell. In this concept the cell is used as a laboratory to perform complex biological operations. Micro- and even nanotechnological tools are employed to access and analyse this laboratory and interface it with the outside world. In the present manuscript we will summarize our recent efforts to demonstrate the advantages of LOC technology to study cells for clinical diagnostics by working on a fertility chip as an example.

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