8. Application Of Pharmacogenetics In Dose Individualization In Diabetes, Psychiatry, Cancer And Cardiology

Dorota Tomalik-Scharte

Department of Pharmacology, University of Cologne , Clinical Pharmacology Unit, Küln, Germany

8.1 Introduction

Following administration of any medication, it is not always possible to predict its effects in the individual patient. Due to the major inter-individual variability in response to pharmacotherapy, in some patients, adverse drug reactions or therapeutic failure instead of therapeutic success are observed. The list of possible factors contributing to the individual drug response involves e.g. age, sex, body weight, liver of kidney function, co-medication or smoking status. Moreover, inter-individual differences in the efficacy and toxicity of many drugs could also be affected by polymorphisms (sequence variants) in genes encoding drug-metabolizing enzymes, transporters, receptors and molecules of signal transduction cascades. Such polymorphisms may contribute to pronounced variability in pharmacokinetic processes (absorption, distribution, metabolism and elimination) and pharmacodynamic effects which finally results in differing drug response. Pharmacogenetics/pharmacogenomics tries to define the influence of genetic variations on drug efficacy and adverse drug reactions. Although both terms are often used interchangeably, pharmacogenetics concentrates on individual drug effects having regard to one or a few gene polymorphisms only, whereas pharmacogenomics assumes application of modern genomic technologies for drug assessment and discovery taking into account the entire genome.

The importance of genetic variations in drug response was recognized about 50 years ago, when in some individuals, live threatening adverse drug reactions following application of the muscle relaxant succinylcholine were observed and in patients treated with the tuberculostatic drug isoniazid, pronounced differences in pharmacokinetic parameters (bimodal distribution) were measured. Later, it was determined that these prime examples of variable drug disposition were caused by inherited differences in genes coding respective drug metabolizing enzymes. Since that time, contribution of genetic polymorphisms in drug metabolizing enzymes, transporters and targets (e.g. receptors) to drug disposition and/or drug effects has been investigated in numerous in vitro and clinical studies. Although more prospective studies with clinical endpoints are required to establish a definite role of molecular genetic diagnostics in individually tailored pharmacotherapy, in many situations pharmacogenetics/pharmacogenomics allows for an improved drug response, yet. Possibilities of individual dose adjustment in some important medical fields are briefly discussed below.

8.2 Diabetes

Type 2 diabetes is one of the most important public health problems and its complications like angio- and neuropathy are associated with pronounced morbidity and

mortality. In addition to lifestyle modification programs, an appropriate therapy with oral antidiabetic drugs plays a key role in blood glucose control. Several classes of antidiabetics such as sulfonylureas, meglitinides, biguanides, a-glucosidase inhibitors, thiazolidinediones or insulins belong to the approved drugs for patients with type 2 diabetes. The action of oral antidiabetic drugs and their adverse drug reactions such as hypoglycemia are subject to wide inter-individual variability. Most oral antidiabetic drugs are metabolized with participation of cytochrome P450 enzymes of the class 2C, which is genetically polymorphic. Whereas sulfonylureas are mostly CYP2C9 substrates, CYP2C8 is the main enzyme responsible for the biotransformation of thiazolidinediones (rosiglitazone and pioglitazone) and repaglinide. For tolbutamide, an oral sulfonylurea hypoglycemic agent used in the treatment of type 2 diabetes for many years, the contribution of CYP2C9 genetic polymorphisms to pharmacokinetics and blood glucose lowering effects was very well documented. Consequently, a careful monitoring of the hypoglycemic effects upon tolbutamide administration in patients heterozygous and especially those homozygous for CYP2C9*3, which is an allele with decreased enzymatic activity, was recommended. Moreover, dose adjustments for carriers of CYP2C9*3 polymorphism were suggested i.e. half and 20% of tolbutamide standard dose, respectively, for heterozygous and homozygous carriers of CYP2C9*3. The impact of CYP2C9 polymorphism on pharmacokinetics of the second generation sulfonylurea drugs like glibenclamide (glyburide), glimepiride and glipizide have also been studied. Similarly, it could have been shown that total clearance of these oral antidiabetics in carriers of CYP2C9*3/*3 genotype was only about 20% of that in wild types (CYP2C9*1/*1), whereas in heterozygotes, this parameter was reduced to 50-80%. Interestingly, the resulting magnitude of differences in drug effects (insulin concentrations) seems to be much less pronounced than for the pharmacokinetic parameters. Nevertheless, it has been considered that respective CYP2C9 genotype-based dose adjustments may reduce the incidence of possible adverse reactions. At the same time, the presence of another common CYP2C9 variant allele i.e. CYP2C9*2 seems to be without clinical relevance for the therapy with sulfonylureas since it has been considered to reduce the CYP2C9 enzymatic activity to a minor extent only.

Both nateglinide and repaglinide are meglitinides, which, like sulfonylureas, act by stimulating insulin release from beta cells of the pancreas via ATP-sensitive K+ channels and on voltage-sensitive Ca 2+ channels. For nateglinide, predominantly metabolized via CYP2C9, it could be shown that CYP2C9*3 polymorphism, but not CYP2C9*2, has a moderate impact on pharmacokinetics and pharmacodynamic effects of the drug in healthy volunteers. Furthermore, following administration of repaglinide, which is metabolized via CYP2C8, reduced plasma concentrations have been determined in carriers of CYP2C8*3 variant allele. The possible role of CYP2C8*3 polymorphism in pharmacokinetics of thiazolidinediones rosiglitazon and pioglitazone should be assessed in further clinical studies.

Biguanide metformin belongs to oral antidiabetics widely used in overweight patients with type 2 diabetes. It could be shown that organic cation transporter 1 (OCT1) is mainly responsible for metformin entry into enterocytes and hepatocytes. To date, several genetic polymorphisms in OCT1, some of them leading to reduced transporter activity, have been identified. In one clinical study, carriers of at least one OCT1 variant allele, determining reduced function of the transporter, showed higher glucose levels following administration of metformin. However, before OCT1 genotyping could be

established as a reliable method for prediction of clinical response to metformin, prospective clinical studies in large numbers of patients must be performed.

It appears that personalized medicine could promise an optimization of treatment choices in patients with type 2 diabetes, however, due to pronounced complexity of the disease and individual drug response, further research is needed to establish the role of pharmacogenetics in therapy of diabetes.

8.3 Psychiatry

Major psychiatric disorders, endogenous depression and schizophrenia, often require a life-long medication with drugs characterized by a narrow therapeutic index and wide inter-individual variability in therapeutic response. Moreover, it is estimated that about 30-50% of patients treated with antidepressants and antipsychotics do not respond sufficiently to the first treatment given to them, which imposes significant costs on public health services. It is expected that identification of genetic factors determining individual drug response in psychiatric disorders could notably improve therapeutic outcomes.

Most antidepressants from the group of tricyclic antidepressants are metabolized with participation of CYP2D6, which is characterized by a high inter-individual variability in catalytic activity mainly determined by the number of functional CYP2D6 alleles. Carriers of two, one or none functional copies of the gene are phenotypically extensive (rapid), intermediate or poor metabolizers, respectively. Furthermore, inheritance of three or more functional alleles by gene duplication or gene amplification determines the ultrafast metabolizer phenotype characterized by higher-than-average enzymatic activity. Tricyclic antidepressants undergo similar biotransformation reactions in the liver, whereas hydroxylation reactions are catalyzed by CYP2D6. For a number of common tricyclics like amitriptyline, clomipramine, desipramine, imipramine, nortriptyline, doxepin and trimipramine, large differences in the pharmacokinetic data depending on CYP2D6 genotype have been documented, so that in poor metabolizers of CYP2D6, reduced (50% or more) clearance values have been observed. On the other hand, following the administration of nortriptyline and desipramine, extremely high clearance was measured in ultrarapid metabolizers of CYP2D6. In addition, CYP2C19, another genetically polymorphic enzyme, can also contribute to metabolism (demethylation) of some tricyclics like imipramine, amitriptyline and clomipramine, however, a possible impact of CYP2C19 polymorphism on the pharmacokinetics of the drugs is not so well documented as that of CYP2D6. Furthermore, CYP2D6 also plays a role in metabolism of another class of antidepressants, i.e. selective serotonine re-uptake inhibitors (SSRIs) and some of them like fluoxetine, fluvoxamine and paroxetine were shown to be strong inhibitors of CYP2D6 activity. For that reason, conversion from extensive to slow and from ultrafast to extensive metabolizer phenotype in course of the therapy with the drugs has been observed. Therefore, for SSRIs, the problem of CYP2D6 inhibition appears to be more relevant than CYP2D6 genetic polymorphisms.

Unfortunately, the data considering potential clinical implications of CYP2D6 genotype in patients treated with antidepressants is very limited, but it seems that poor metabolizers of CYP2D6 tend to be more affected by relevant adverse effects, whereas

the role of CYP2D6 in response to antidepressants is rather controversial.

CYP2D6 polymorphisms can also affect the pharmacokinetic parameters of commonly prescribed conventional as well as atypical neuroleptics like haloperidol, levomepromazine, perazine, thioridazine, clozapine, olanzpaine or risperidone. Moreover, CYP2D6 genotype has been associated with an increased risk of antipsychotic-induced extrapyramidal symptoms, which frequently accompany the therapy with conventional antipsychotics. For haloperidol, pseudoparkinsonic adverse events were significantly more frequent in poor metabolizers of CYP2D6, whereas with a higher number of active CYP2D6 gene copies, a tendency toward a lower therapeutic efficacy was observed.

For some antidepressants and neuroleptics, possible dose adjustments have been calculated on the base of CYP2D6 and CYP2C19 genotypes. In carriers of CYP2D6-related poor metabolizer genotype, dose reductions to about one third of the standard dose have been suggested for drugs like tricyclics impiramine, trimipramine, doxepin or antipsychotic drug perphenazine, to name a few examples. At the same time, dose enhancements by about one third of the standard treatment for extensive metabolizers were calculated for these drugs. Likewise, dose extrapolations resulting from CYP2C19-mediated quantitative influences on pharmacokinetics of some antidepressant drugs are possible. Notably, assessment of both genes CYP2D6 and CYP2C19 has found the way into clinical practice by means of the recent approval of the respective pharmacogenetic tests by the Food and Drug Administration.

As genetic polymorphisms in genes coding for drug metabolizing enzymes can explain only a part of the large inter-individual variability in therapeutic response in psychiatric disorders, other candidate genes which code for target molecules should also be considered. However, data on the possible medical impact of the particular polymorphisms affecting targets like neuronal serotonin transporter, serotonin and dopamine receptors as well as several molecules of signal transduction are not so well documented or partially controversial, so that conclusive clinical evidence is missing in many cases and no respective treatment recommendations are possible at present.

In summary, there is a strong evidence first of all for CYP2D6 genotype affecting pharmacokinetics of numerous antidepressants and antipsychotic drugs and respective dose extrapolations for carriers of genetic polymorphisms have been calculated. However, before dose individualization based on genotype could be routineously implemented in clinical practice, it should firstly be validated in prospective and controlled clinical studies.

8.4 Oncology

Application of pharmacogenetics to individualization of therapy with antineoplastic drugs, most of them characterized by a narrow therapeutic index and life-threatening adverse reactions, seems to promise improvement of drug effects in some cases.

Thiopurines, like 6-mercaptopurine and thioguanine, largely used in the treatment of acute leukemia, are one of the earliest examples of importance of pharmacogenetics in individualized drug therapy. Following the activation to thioguanine nucleotides via the

purine salvage pathway and incorporation into DNA as false purine bases, they are metabolized by the enzyme thiopurine-S-methyltransferase (TPMT) to inactive compounds. The individual enzymatic capacity is a subject to large inter-individual variability which is determined by genetic polymorphisms, with three variant alleles *2, *3A and *3C explaining about 80-95% of enzymatic deficiency. In the Caucasian population, about 89% of people exhibit a high TPMT activity, whereas in 11 and 0.3% of individuals, respectively, intermediate and low activity, is observed. Following a treatment with conventional doses of thiopurines, patients showing diminished catalytic TPMT activity are at increased risk of bone marrow suppression, which may result in fatal outcomes and require discontinuation of therapy. Hepatic TPMT activity can be reliably determined by genotyping or measurement of the catalytic activity of cytosolic TPMT in erythrocytes using established radiochemical or HPLC methods (i.e. phenotyping). Measurement of TPMT activity should routinely precede onset of therapy with thiopurine-derived drugs in order to minimize myelotoxic adverse events. For patients being carriers of two non-functional TPMT, thiopurine dose reduction to 5-10% of standard dose was recommended to allow for an efficacious therapy. In heterozygous patients, the therapy begins with a full dose, but a subsequent dose reduction may be required. Although only a small percentage of patients could be affected by inherited differences in TPMT activity, the clinical consequences may be crucial. For that reason the Food and Drug Administration has already implemented respective pharmacogenetic data into the product label of 6-mercaptopurine, widely used for childhood leukemia.

Another antineoplastic drug for which pharmacogenetic diagnostics prior to therapy onset would promise selection of potentially toxic patients is 5-Fluorouracil (5-FU). Dihydropyrimidine dehydrogenase (DPD) is a key enzyme in the hepatic metabolism of 5-FU and its derivatives such as capecitabine, so that the enzyme activity affects pharmacokinetics, efficacy, and toxicity of the drugs. Diminished enzymatic activity has been observed in about 3-5% of Caucasians and can potentially result in severe adverse drug reactions like mucositis or granulocytopenia in cancer patients treated with 5-FU. DPD is genetically polymorphic and allelic variants in the gene coding the enzyme have been associated with reduced catalytic activity. One of the best described mutations is the the so-called exon 14-skipping mutation at the 5'-splice donor site of exon 14. Although this polymorphism is present in only about 1% of Caucasians, it has been detected in 24% of patients developing severe toxicity (WHO grade IV) following treatment with 5-FU. Nevertheless, further research is needed to evaluate possible benefits of pharmacogenetic strategies upon therapy with 5-FU.

At the same time, pharmacogenetics of irinotecan, a potent antineoplastic agent used in the treatment of colorectal cancer and small-cell lung cancer, seems to be one of few promising examples of the implementation of pharmacogenetics to individualized drug therapy. Following its application, irinotecan is metabolized to the active compound SN-38, which is a topoisomerase I inhibitor. In the next step, SN-38 is glucuronidated to its inactive form by various isoenzymes of uridine diphosphate glucuronosyltransferase (UGT), first of all UGT1A1, which is also responsible for glucuronidation of bilirubin. Reduced glucuronidation activity of the UGT1A1 enzyme has been connected to elevated levels of SN-38 and toxic effects like severe diarrhea and neutropenia in patients treated with irinotecan. To date, several genetic polymorphisms leading to impaired UGT1A1 activity have been determined in the gene coding for the enzyme. In the Caucasian population, the UGT1A1*28 polymorphism (TA repeat in the promoter

region) is the most frequent variant contributing to reduced glucuronidation activity. It could be shown that even in heterozygous carriers of the variant allele, pronounced changes in irinotecan disposition and severe toxicity occur. For that reason, genotyping for UGT1A1 polymorphisms before the onset of ironotecan therapy has been recommended. Interestingly, the measurement of total bilirubin level seems to be an easy surrogate parameter, if genotyping is not possible. Patients with diminished glucuronidation capacity should be administered a reduced initial dose of irinotecan to avoid the above mentioned severe toxicities.

Possible implications of polymorphisms in genes coding for other drug metabolizing enzymes like CYP2D6 and CYP3A, drug transporters like ATP-binding cassette transporter ABCB1 (P-glycoprotein) and drug targets like thymidylate synthase in patients treated with common prescribed antineoplastic drugs have also been considered in numerous studies, but their potential impact on clinical outcomes is still controversial.

In summary, oncology is the clinical area where achievements of modern pharmacogenomic diagnostics have already been used to tailor individual therapy with some antineoplastic drugs, but for a wide implementation of genotyping in cancer patients, more clinical data and a precise cost effectiveness analysis of this approach are required.

8.5 Cardiology

Cardiovascular diseases like coronary heart disease, hypertension or heart failure are still a leading health problem in developed countries and respective pharmacotherapy is an established approach in affected patients. It appears that pharmacogenetics throws some new light on the question of treatment amendment with respect to cardiovascular diseases.

For several beta-blockers, which belong to the most often prescribed drugs in patients with cardiovascular diseases, possible effects of genetic polymorphisms in drug metabolizing enzymes like CYP2D6 were assessed. CYP2D6 is the key enzyme in metabolism of metoprolol and pronounced differences between CYP2D6 extensive and rapid metabolizers with respect to the phramacokinetics of the drug have been observed. Moreover, *CYP2D6* polymorphism has been shown to contribute to pharmacodynamic response following the administration of metoprolol, since reduction of exercise induced heart rate by the drug in the group of ultra rapid metabolizers (carrying a duplication of the *CYP2D6* gene) was only circa half of that observed in extensive metabolizers. Also for carvedilol, the role of the CYP2D6 polymorphism was studied. However, respective pharmacokinetic differences resulted from the genetic polymorphism seem to be without any effects on heart rate and blood pressure so that they will have no clinical significance.

Another class of drugs, AT 1 (angiotensin II type 1) receptor antagonists (sartans), used to treat hypertension or heart failure, could be potential candidate for consideration of pharmacogenetic data in therapy optimization. Most sartans are metabolized with participation of genetically polymorphic CYP2C9. Losartan is a pro-drug which is transformed to its active form, i.e. E-3174, via CYP2C9 and CYP3A4. Unfortunately the role of the *CYP2C9* polymorphism for therapy with losartan is quite controversial.

Whereas in one study, presence of *CYP2C9*3* was shown to be associated with decreased formation of E-3174, in another study, no differences with respect to the pharmacokinetics of the parent drug and its active metabolite between the wild types and carriers of the best investigated *CYP2C9* variant alleles related to impaired intrinsic enzymatic activity *CYP2C9*3 and CYP2C9*2* were determined. There is also some clinical data suggesting the role of *CYP2C9* polymorphism in the pharmacokinetics and/or -dynamics of other AT 1 receptor antagonists like irbesartan or candesartan. However, if potential dose adjustment of sartans according to the *CYP2C9* genotype might be beneficial is furthermore doubtful.

Recently, importance of pharmacogenetic implications has also been discussed for statins (HMG-CoA reductase inhibitors), administered to lower cholesterol level in numerous patients with or at risk for cardiovascular problems. Statins are the most prescribed and most effective drugs in lipid lowering therapy but large variability in response is observed and in nearly one of three patients treatment goals could not be met. It has been reported that in patients treated with pravastatin, cholesterol lowering effects are poorer in carriers of two common and tightly linked single nucleotide polymorphisms localized in the gene coding for HMG-CoA reductase, which is the target enzyme for statin therapy. However, no data is available, if possible genotyping approach with a following dose adjustment, in terms of application of a higher dose of pravastatin in patients carrying the variant haplotype, could be advantageous in clinical practice.

Last but not least, the meaning of pharmacogenetic approaches for therapy with oral anticoagulants (coumarin anticoagulants) should be briefly discussed. These vitamin K antagonists, used widely in patients at risk of thromboembolic disorders, are characterized by a narrow therapeutic index, so that the therapy with them is often complicated by dangerous bleeding episodes or lack of efficacy, in case of under- or overcoagulation, respectively. Two polymorphic genes, *CYP2C9* and vitamin K epoxide reductase complex subunit 1 (*VKORC1*), can contribute significantly to the known inter-individual variability in the effectiveness of oral anticoagulants. The role of the enzyme CYP2C9 in metabolism of the warfarin and its analogues acenocoumarol and phenprocoumon is well documented. The variant alleles with decreased enzymatic activity *CYP2C9* *2 and *CYP2C9* *3 have been demonstrated to impact considerably the pharmacokinetics of S-warfarin (which is 3 to 5 times more potent than the R-isomer) and so to influence the antithrombotic activity of the drug. Patients carrying at least one variant allele, show a longer induction period to achieve a stable warfarin dosing and tend to have increased values of international normalized ratio (INR). They are also at increased risk of life threatening bleedings. Similarly, there is a good evidence for the role of *CYP2C9* polymorphism in the anticoagulation effects of acenocoumarol and phenprocoumon in the literature data. For that reason, *CYP2C9* genotyping was suggested as a useful approach to select a population of patients who are potentially at risk of complications associated with oral anticoagulants and who may require a reduced dose of the drugs.

VKORC1 is the target molecule of vitamin K antagonists and polymorphisms in *VKORC1* gene, in addition to *CYP2C9* and demographic factors, seem to explain a significant part of the inter-individual variability in pharmacokinetics and dynamics of the drugs and consequently could be essential for determination of the individual dose. For warfarin, an algorithm for individual dosing adjustment on the base of *CYP2C9* and

VKORC1 genotype, age and height has been proposed, but prior to introduction into clinical practice it should be proved in prospective clinical studies.

In summary, in the light of current knowledge, it seems that with respect to cardiovascular diseases, only for vitamin K antagonists, there is a place for pharmacogenetic approaches to optimize the therapy and avoid adverse events.

8.6 Conclusion

Looking back at more than 50 years of pharmacogenetic experience, we have learnt that an important part of the inter-individual variability in drug response is caused by polymorphisms in drug metabolizing enzymes, transporters or target molecules. For some treatments, it was shown that efficacy and safety profile of pharmacotherapy could be improved if respective allelic variations are taken into account. Although it seems that the first genotype-specific dose recommendations have already reached clinical practice in some medical fields, unquestionably more prospective clinical studies validating pharmacogenetic approaches as well as cost-effectiveness evaluations are needed before pharmacogenetics makes a great jump form bench to bedside.

Recommended literature :

1 Kirchheiner J, Fuhr U, Brockmüller J. Pharmacogenetics-based therapeutic recommendations--ready for clinica l practice? Nat Rev Drug Discov 2005;4:639-47.

2 Tomalik-Scharte D, Lazar A, Fuhr U, Kirchheiner J. The clinical role of genetic polymorphisms in drug-metabolizing enzymes. Pharmacogenomics J. 2007.

3 Kirchheiner J, Roots I, Goldammer M, Rosenkranz B, Brockmüller J. Effect of genetic polymorphisms in cytochrome p450 (CYP) 2C9 and CYP2C8 on thepharmacokinetics of oral antidiabetic drugs: clinica l relevance. Clin Pharmacokinet 2005;44(12):1209-25.

4 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; 9:442-73.