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TOOLS IN PHARMACOVIGILANCE IN PSYCHIATRY: THERAPEUTIC DRUG MONITORING, PHARMACOGENETIC TESTS AND DRUG-DRUG INTERACTIONS DATABASES

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Content

List of Abbreviations

5HT1a	Serotonin 1a receptor
5HT2a	Serotonin 2a receptor
5HT2c	Serotonin 2c receptor
5HT6	Serotonin 6 receptor
5HT7	Serotonin 7 receptor
α1	α1 receptor
α2	α2 receptor
ABCB1	ATP-binding cassette sub-family B member 1, also called P-glycoprotein or MDR1
ADE	Adverse Drug Event
ADME	Absorption, Distribution, Metabolism, Excretion
ADR	Adverse Drug Reaction
AGNP	Arbeitsgemeinschaft für Neuropsychopharmakologie und Pharmakopsychiatrie
AMSP	Arzneimittelsicherheit in der Psychiatrie
AMÜP	Arzneimittelüberwachung in der Psychiatrie
ATP	Adenosine triphosphate
BMI	Body mass index
CHMP	Committee for Medicinal Products for Human Use
CYP	Cytochrome
CYP450	Cytochrome P450
D2	Dopamine 2 receptor
D3	Dopamine 3 receptor
D4	Dopamine 4 receptor
DDI	Drug- drug interaction
DNA	Deoxyribonucleic acid
ECG	Electrocardiogram
EDTA	Ethylenediaminetetraacetic acid

EEG	Electroencephalogram
EM	Extensive metaboliser
EMEA/EMA	European Medicines Agency (newer abbreviation is EMA)
EPS	Extrapyramidal symptoms
FDA	Food and Drug Administration
FMO3	Flavin containing monooxygenase 3
H1	Histamine 1 receptor
H3	Histamine 3 receptor
HIV	Human immunodeficiency virus
ICD	International classification of diseases
ICH	International Conference on Harmonization (see http://www.ich.org/home.html)
IM	Intermediate metaboliser
M1	Muscarinic acetylcholine receptor 1
M2	Muscarinic acetylcholine receptor 2
M3	Muscarinic acetylcholine receptor 3
M4	Muscarinic acetylcholine receptor 4
M5	Muscarinic acetylcholine receptor 5
MAO	Monoamino oxidase
MAOI	Monoamino oxidase inhibitor
MDR	Multi drug resistance
mediQ	Qualitätszentrum für Medikamentensicherheit und Diagnostik, created the interaction program mediQ.ch (often named mediQ)
MRI	Magnetic resonance imaging
mut	Mutant
NAT	N-acteyltransferase
OATP	Organic anion-transporting peptide
ODV	O-desmethylvenlafaxine
PCR	Polymerase chain reaction

Pgp	P-glycoprotein = permeability glycoprotein
PM	Poor metaboliser
SADR	Serious Adverse Drug Reaction
SPC	Summary of Product Characteristics
SRI	Serotonin reuptake inhibitor
SSRI	Selective serotonin reuptake inhibitor
TDM	Therapeutic Drug Monitoring
UGT	UDP-glucuronosyltransferase
UM	ultrarapid metaboliser
USA	United States of America
wt	Wild type
WHO	World Health Organization

1 Summary and Acknowledgements

Summary

In order to increase drug safety we must better understand how medication interacts with the body of our patients and this knowledge should be made easily available for the clinicians prescribing the medication. This thesis contributes to how the knowledge of some drug properties can increase and how to make information readily accessible for the medical professionals. Furthermore it investigates the use of Therapeutic drug monitoring, drug interaction databases and pharmacogenetic tests in pharmacovigilance.

Two pharmacogenetic studies in the naturalistic setting of psychiatric in-patients clinics have been performed; one with the antidepressant mirtazapine, the other with the antipsychotic clozapine. Forty-five depressed patients have been treated with mirtazapine and were followed for 8 weeks. The therapeutic effect was as seen in other previous studies. Enantioselective analyses could confirm an influence of age, gender and smoking in the pharmacokinetics of mirtazapine; it showed a significant influence of the *CYP2D6* genotype on the antidepressant effective S-enantiomer, and for the first time an influence of the *CYP2B6* genotype on the plasma concentrations of the 8-OH metabolite was found. The *CYP2B6*/*6* genotype was associated to better treatment response. A detailed hypothesis of the metabolic pathways of mirtazapine is proposed. In the second pharmacogenetic study, analyses of 75 schizophrenic patients treated with clozapine showed the influence of *CYP450* and *ABCB1* genotypes on its pharmacokinetics. For the first time we could demonstrate an in vivo effect of the *CYP2C19* genotype and an influence of P-glycoprotein on the plasma concentrations of clozapine. Further we confirmed in vivo the prominent role of CYP1A2 in the metabolism of clozapine.

Identifying risk factors for the occurrence of serious adverse drug reactions (SADR) would allow a more individualized and safer drug therapy. SADR are rare events and therefore difficult to study. We tested the feasibility of a nested matched case-control study to examine the influence of high drug plasma levels and *CYP2D6* genotypes on the risk to experience an SADR. In our sample we compared 62 SADR cases with 82 controls; both groups were psychiatric patients from the in-patient clinic Königsfelden. Drug plasma levels of >120% of the upper recommended references could be identified as a risk factor with a statistically significant odds ratio of 3.5, a similar trend could be seen for CYP2D6 poor metaboliser. Although a matched case-control design seems a valid method, 100% matching is not easy to perform in a relative small cohort of one in-patient clinic. However, a nested case-control study is feasible.

On the base of the experience gained in the AMSP+ study and the fact that we have today only sparse data indicating that routine drug plasma concentration monitoring and/or pharmacogenetic testing in

psychiatry are justified to minimize the risk for ADR, we developed a test algorithm named “TDM plus” (TDM plus interaction checks plus pharmacogenetic testing).

Pharmacovigilance programs such as the AMSP project (AMSP = Arzneimittelsicherheit in der Psychiatrie) survey psychiatric in-patients in order to collect SADR and to detect new safety signals. Case reports of such SADR are, although anecdotal, valuable to illustrate rare clinical events and sometimes confirm theoretical assumptions of e.g. drug interactions. Seven pharmacovigilance case reports are summarized in this thesis.

To provide clinicians with meaningful information on the risk of drug combinations, during the course of this thesis the internet based drug interaction program mediQ.ch (in German) has been developed. Risk estimation is based on published clinical and pharmacological information of single drugs and alimentary products, including adverse drug reaction profiles. Information on risk factors such as renal and hepatic insufficiency and specific genotypes are given. More than 20’000 drug pairs have been described in detail. Over 2000 substances with their metabolic and transport pathways are included and all information is referenced with links to the published scientific literature or other information sources. Medical professionals of more than 100 hospitals and 300 individual practitioners do consult mediQ.ch regularly. Validations with comparisons to other drug interaction programs show good results.

Finally, therapeutic drug monitoring, drug interaction programs and pharmacogenetic tests are helpful tools in pharmacovigilance and should, in absence of sufficient routine tests supporting data, be used as proposed in our TDM plus algorithm.

Résumé

Pour améliorer la sécurité d'emploi des médicaments il est important de mieux comprendre leurs interactions dans le corps des patients. Ensuite le clinicien qui prescrit une pharmacothérapie doit avoir un accès simple à ces informations. Entre autres, cette thèse contribue à mieux connaître les caractéristiques pharmacocinétiques de deux médicaments. Elle examine aussi l'utilisation de trois outils en pharmacovigilance : le monitorage thérapeutique des taux plasmatiques des médicaments (« therapeutic drug monitoring »), un programme informatisé d'estimation du risque de combinaisons médicamenteuses, et enfin des tests pharmacogénétiques.

Deux études cliniques pharmacogénétiques ont été conduites dans le cadre habituel de clinique psychiatrique : l'une avec la mirtazapine (antidépresseur), l'autre avec la clozapine (antipsychotique). On a traité 45 patients dépressifs avec de la mirtazapine pendant 8 semaines. L'effet thérapeutique était semblable à celui des études précédentes. Nous avons confirmé l'influence de l'âge et du sexe sur la pharmacocinétique de la mirtazapine et la différence dans les concentrations plasmatiques entre fumeurs et non-fumeurs. Au moyen d'analyses énantiomères sélectives, nous avons pu montrer une influence significative du génotype *CYP2D6* sur l'énantiomère S+, principalement responsable de l'effet antidépresseur. Pour la première fois, nous avons trouvé une influence du génotype *CYP2B6* sur les taux plasmatiques de la 8-OH-mirtazapine. Par ailleurs, le génotype *CYP2B6*6/*6* était associé à une meilleure réponse thérapeutique. Une hypothèse sur les voies métaboliques détaillées de la mirtazapine est proposée. Dans la deuxième étude, 75 patients schizophrènes traités avec de la clozapine ont été examinés pour étudier l'influence des génotypes des iso-enzymes CYP450 et de la protéine de transport ABCB1 sur la pharmacocinétique de cet antipsychotique. Pour la première fois, on a montré in vivo un effet des génotypes *CYP2C19* et *ABCB1* sur les taux plasmatiques de la clozapine. L'importance du CYP1A2 dans le métabolisme de la clozapine a été confirmée.

L'identification de facteurs de risques dans la survenue d'effets secondaire graves permettrait une thérapie plus individualisée et plus sûre. Les effets secondaires graves sont rares. Dans une étude de faisabilité (« nested matched case-control design » = étude avec appariement) nous avons comparé des patients avec effets secondaires graves à des patients-contrôles prenant le même type de médicaments mais sans effets secondaires graves. Des taux plasmatiques supérieurs à 120% de la valeur de référence haute sont associés à un risque avec « odds ratio » significatif de 3.5. Une tendance similaire est apparue pour le génotype du CYP2D6. Le « nested matched case-control design » semble une méthode valide qui présente cependant une difficulté : trouver des patients-contrôles dans le cadre d'une seule clinique psychiatrique. Par contre la conduite d'une « nested case-control study » sans appariement est recommandable.

Sur la base de notre expérience de l'étude AMSP+ et le fait que nous disposons que de peux de données justifiant des monitorings de taux plasmatiques et/ou de tests pharmacogénétiques de routine, nous avons développé un test algorithme nommé « TDMplus » (TDM + vérification d'interactions médicamenteuses + tests pharmacogénétique).

Des programmes de pharmacovigilances comme celui de l'AMSP (Arzneimittelsicherheit in der Psychiatrie = pharmacovigilance en psychiatrie) collectent les effets secondaires graves chez les patients psychiatriques hospitalisés pour identifier des signaux d'alertes. La publication de certains de ces cas même anecdotiques est précieuse. Elle décrit des événements rares et quelques fois une hypothèse sur le potentiel d'une interaction médicamenteuse peut ainsi être confirmée. Sept publications de cas sont résumées ici.

Dans le cadre de cette thèse, on a développé un programme informatisé sur internet (en allemand) – mediQ.ch - pour estimer le potentiel de risques d'une interaction médicamenteuse afin d'offrir en ligne ces informations utiles aux cliniciens. Les estimations de risques sont fondées sur des informations cliniques (y compris les profils d'effets secondaires) et pharmacologiques pour chaque médicament ou substance combinés. Le programme donne aussi des informations sur les facteurs de risques comme l'insuffisance rénale et hépatique et certains génotypes. Actuellement il décrit en détail les interactions potentielles de plus de 20'000 paires de médicaments, et celles de 2000 substances actives avec leurs voies de métabolisation et de transport. Chaque information mentionne sa source d'origine; un lien hypertexte permet d'y accéder. Le programme mediQ.ch est régulièrement consulté par les cliniciens de 100 hôpitaux et par 300 praticiens indépendants. Les premières validations et comparaisons avec d'autres programmes sur les interactions médicamenteuses montrent de bons résultats.

En conclusion : le monitorage thérapeutique des médicaments, les programmes informatisés contenant l'information sur le potentiel d'interaction médicamenteuse et les tests pharmacogénétiques sont de précieux outils en pharmacovigilance. Nous proposons de les utiliser en respectant l'algorithme « TDM plus » que nous avons développé.

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2 Aims

“Pharmacovigilance is the science and activities relating to detection, assessment, understanding, and prevention of adverse effects or any drug-related problems “

(World Health Organisation 2002)

The main and general aim of this thesis is a contribution towards improving drug safety for the psychiatric patient by finding means to minimize the risks of his or her drug treatment. Drug safety in psychiatry requires special attention: on the one hand, drugs are administered to patients who cannot always communicate their symptoms and who are sometimes treated against their will and in pressing situations of emergency, often involving polypharmacy and high doses. On the other hand, the pharmacology of psychotropic drugs and their interactions in drug combinations are still insufficiently known, as are the biological foundations of the diseases to be treated.

In order to minimize risks, we have to be aware of them. Knowledge about drug characteristics and interactions with patient factors has to improve. To this goal, we performed two clinical studies in order to increase the clinically relevant pharmacokinetic knowledge about the antidepressant mirtazapine and the antipsychotic clozapine.

Therapeutic Drug Monitoring, drug interaction checking programs and pharmacogenetic tests are in 2011 considered to be useful tools for clinicians to minimize risks of drug therapies and help in causality assessment in pharmacovigilance. However, at the preparation of this thesis in 2001 clinicians hardly used or even knew these tools. We implemented the use of Therapeutic Drug Monitoring (TDM) and pharmacogenetic tests in a clinical setting and in a case-control study evaluated the relationship between drug plasma levels, CYP2D6 genotypes and the risk of experiencing a serious adverse drug reaction.

Furthermore, we developed a user-friendly drug interaction database that estimates the risk of drug combinations by taking into account pharmacokinetic and pharmacodynamic properties of the combined substances and some patient-related risk factors such as pharmacogenetics, diet and lifestyle.

This thesis is structured as follows:

Introduction (chapter 3)

In chapter 2 general considerations about pharmacovigilance in psychiatry and, more specifically, the drug safety project AMSP (*Arzneimittelsicherheit in der Psychiatrie*) will be presented, followed by an introduction to TDM, pharmacogenetics and drug-drug interactions.

The pharmacogenetics of mirtazapine and clozapine (chapters 4 and 5)

In chapters 4 and 5 the clinical trials with mirtazapine, in depressed, and clozapine, in schizophrenic patients, are presented, which examine the influence of pharmacogenetics and other factors on their pharmacokinetics and subsequent clinical consequences. Therapeutic drug monitoring and pharmacogenetic tests (genotyping and phenotyping) were used in connection with a clinical study protocol. These protocols, moreover, mimic the normal practice of a psychiatric in-patient clinic.

Feasibility study AMSP+: a nested case-control study with psychiatric inpatients (chapter 6)

Within the international quality assurance and research project AMSP the feasibility of using therapeutic drug monitoring and pharmacogenetic tests in the causality assessment of serious adverse reactions has been evaluated. In addition, a pilot nested case-control study was set up to test the feasibility of this design for a larger study examining the relationship between plasma levels and genotypes and the risk of developing a serious adverse drug reaction.

Web-based drug interactions database: mediQ.ch (chapter 7)

Risk assessment in drug combination therapy is often very complex and time-consuming and, when this thesis was still in its preparatory phase in 2001, it was hardly part of clinical routine at all. Major risks were taken and serious adverse reactions resulted which could be have been avoided if such knowledge had been available more easily. A major part of this thesis consisted of creating an accurate and user-friendly drug interaction program offering clinicians this knowledge in an easily accessible and timely manner, always keeping in mind the time constraints of the clinical work context.

Pharmacovigilance case studies (chapter 8)

Anecdotal information from well-documented individual cases can be valuable for the detection of rare adverse drug effects and also for didactic purposes. A selection of such cases is presented in chapter 8.

Concluding remarks and outlook (chapter 9)

Chapter 9, finally, looks back and summarizes the contributions of this thesis to improve drug safety for the psychiatric patient and provides an outlook on further activities planned in relation to this project.

3 Introduction

Not only effective but also safe and well tolerated medication is one of the major goals of today's drug development. In spite of all these efforts the number of serious and life threatening adverse drug reactions seems not to decrease. A meta-analysis from 1998 (1) showed an incidence of 6.7% serious adverse drug reactions (SADR), whereas fatal reactions involved 0.32% of hospitalized patients; calculated for the population of the United States of America (USA) meaning that about 2.2 Mio in-patients suffer annually from SADR and 100 000 die from it. A study from the Food and Drug Administration (FDA) in the USA show a 2.6 fold increase in SADR and related deaths over the period from 1998 to 2005 (2). These numbers represent a serious medical and socio-economical problem (3-6).

The majority of Adverse Drug Reactions (ADR) are predictable from the pharmacologic action of the drug (type A reactions, see also table 1) and are therefore considered at least partly avoidable. One strategy is to identify vulnerable individuals by biomarker tests (e.g. pharmacogenetic) (7), by taking into account co-morbidities and life style of the patient, and eventually by checking possible drug interaction potentials. Another strategy is intensified surveillance of the patient during treatment by e.g. Therapeutic Drug Monitoring (TDM), laboratory testing, electrocardiogram (ECG), and others for early detection and intervention to limit the extent of harm. Large pharmacovigilance surveillance programs such as the AMSP project (see page 17) help to detect adverse drug reactions and their risk factors.

Pharmacovigilance in Psychiatry

In psychiatry we are often confronted with chronic diseases requiring long term medication and with increasing polypharmacy (8-10). Polypharmacy is on average 3.5 and 5 drugs in patients < 65 years and 65 years and older, respectively (AMSP (11) data on file, R. Grohmann personal communication). Psychotropic drugs have many side effects but the rate of serious adverse drug reactions seems with 1.5 - 2% (data from the AMSP project) lower than in other medical disciplines with 6-7% (1;3). That could be explained by underreporting or because psychiatric patients are not always easy with expressing their suffering of side effects, and differentiation between adverse drug reactions and symptoms of underlying illness can be difficult. Data of the AMSP project show that a serious adverse drug reaction leads to a doubling of the hospitalisation duration which means a considerable burden, also economically, the average hospitalisation duration increasing from approximately 25 days to 50 days (data from the AMSP project).

Type of Adverse Drug Reactions (12)

When a causal relationship with the drug taken is established, an adverse drug event (ADE) is considered to be an adverse drug reaction (ADR). A serious adverse drug reaction meets one of the generally accepted following criteria: death, life threatening, causing permanent damage, leading to or prolonging hospitalisation (Definitions ICH E2A 1995 Step 5 revised in 2006).

Two major classes of ADR exist: Type A and Type B (13). Type A are common (> 80% of all ADR), predictable, and tend to be dose- or more exactly concentration-related and less serious than those aberrant effects of the Type B reactions. Type A reactions can result from too much of a drug (too high dosage, pharmacokinetic drug interaction, normal dosage but the person metabolises or excretes the drug only very slowly, normal dosage but the person absorbs more drug than common, normal dosage but the person is overly sensitive), but also by pharmacodynamic drug interactions (serotonin syndrome by combining several serotonin agonists) or in response to a secondary drug pharmacology (torsade de pointes in a patient with a long QT syndrome) (Table 1).

Table 1: Classification of adverse drug reactions

Predictable **(also known as Type A)**	**Unpredictable** **(also known as Type B)**

• Overdosage/toxicity: e.g., nephrotoxicity caused by elevated aminoglycoside levels; coma because of elevated benzodiazepine levels
• Side effects: e.g., constipation caused by chronic opiate use
• Secondary or indirect effects
  o related to drug alone: e.g., disturbance of vaginal flora due to antibiotic use
  o related to both disease and drug: e.g., ampicillin rash in association with Epstein- Barr virus
• Drug interactions: e.g., use of terfenadine (now withdrawn from the market) in combination with ketoconazole can result in torsade de pointes caused by elevated terfenadine levels; combination of fluvoxamine and clozapine can results in delirium due to very high clozapine plasma levels
	• Intolerance: e.g., tinnitus caused by small doses of aminosalicylic acid
	• Allergic (hypersensitivity or immunologic): result of an immune response to a drug, e.g., penicillin-induced urticaria
	• Pseudo-allergic (non-immunologic): immediate, generalized reaction involving mast cell mediator release, e.g., respiratory symptoms induced by non-steroidal anti-inflammatory drugs
	• Idiosyncratic: unexpected response to a drug and differing from its pharmacological actions; not related to an allergic mechanism, e.g., anticonvulsant hypersensitivity syndrome reaction (characterized by fever, cutaneous eruption and internal organ involvement)

Since the majority of ADR are type A reactions, which tend to be concentration-dependent, one could hypothesize that (too) high plasma concentrations are a risk factor for an ADR and that by avoiding them a substantial amount of ADR could be prevented. This seems obvious when studying some individual cases but how relevant is this risk in a larger patient population?

The Project „Arzneimittelsicherheit in der Psychiatrie“ AMSP

Legislated pharmacovigilance took its beginning in the early1960ties with the invalidating thalidomide effects on babies of mothers who took this drug against morning sickness during pregnancy. The government of the USA decided to regulate Drug Safety within the FDA. Other countries followed with their own regulatory bodies and legislation, and post marketing surveillance was institutionalized. In 1973 a number of fatal cases of agranulocytosis cases occurred under treatment with clozapine which led to the withdrawal of this efficacious antipsychotic drug in several countries. As a reaction to that, in 1979, the AGNP (Arbeitsgemeinschaft für Neuropsychopharmakologie) founded the pharmacovigilance working group AMÜP (Arzneimittelüberwachung in der Psychiatrie). The AMÜP study was a model for the continuous and systematic post marketing surveillance of psychiatric in-patients collecting data on the nature and frequency of adverse drug reactions in a natural psychiatric setting (14;15). It was performed in the university clinics of Munich, Göttingen and Berlin and was supported by the former Bundesgesundheitsamt (federal office of health) of Germany. It lasted 20 years.

With the methodology and experience of AMÜP the AMSP project was developed from 1990 – 1993 by Hans Hippius, Eckart Rüther, Rolf Engel and Renate Grohmann (16). Since 1993 AMSP surveys around 30’000 beds in approximately 57 psychiatric clinics in Germany, Austria and Switzerland. Serious adverse drug reactions (as defined by the AMSP project, table 2) are collected by AMSP drug monitors (medical doctors) in the naturalistic setting of the psychiatric inpatient clinic.

Causality assessment is made by the drug monitor and the responsible treating doctor according to standard causality assessment criteria:

1. Association in time between drug administration and event,
2. Pharmacology (features, previous knowledge of side effects),
3. Medical plausibility (characteristic sign and symptoms, laboratory assessments, pathological findings), and
4. Likelihood of other causes, risk factors (17).

Table 2: Serious adverse drug reactions according AMSP criteria

Life threatening or fatal, permanent disability, potentially life threatening, severely incapacitating. Examples by organ system:

Psychic ADR: Suicide, suicide attempt, suicidal ideation, delirium, paranoid/hallucinatory or catatonic syndrome, depression, mania, coma, sopor, somnolence, aggressivity, obsessive compulsive symptoms, substance dependency, severe sedation, respiratory depression, frightening nightmares, severe psychomotor agitation,..

Neurological ADR: Incapacitating extrapyramidal-motor symptoms such as severe early dyskinesia, Parkinson syndrome, and akathisia; tardive dyskinesia, malignant neuroleptic syndrome, catatonic neuroleptic syndrome, Rabbit syndrome, atypical dyskinesia such as Pisa syndrome. Furthermore seizures, serotonin syndrome, ataxia, severe myocloni, severe tremor, speaking disorder, tinnitus, severe accommodation disturbances, diplopia, paraesthesia, restless legs.

Cardiovascular ADR: Collapse, heart insufficiency, cardiac arrest, myocardial infarct, myocarditis, deep vein thrombosis, embolia, cerebrovascular disturbances, hypertension > 180/110 mmHg, symptomatic hypotension < 90 mmHg, conduct disorders, arrhythmia, bradycardia < 40/min., tachycardia > 120/min, atriofibrillation, AV-block II and III, QT-interval prolongation > 500ms or increase of > 25%, torsade de pointes, ..

Liver disturbances: Liver value increase > 5 times the norm value, (AST, ALT, Y-GT, AP), severe cholestasis, hepatitis, ..

Gastro-intestinal ADR: Severe vomiting or diarrhoea, severe nausea of longer than week, severe constipation, massive hypersalivation, pancreatitis, subileus and ileus, oesophagitis, ..

Dermatological ADR: Allergic dermatological reactions, severe rash, Quincke oedema, allergic vasculitis, new manifestation or exacerbation of psoriasis, severe acne, severe hair loss, massive oedema, ..

Haematological ADR: Neutropenia < 1500 neutrophiles/mm3 = <1,5/nl and agranulocytosis (< 500 neutrophiles/mm3 = < 0,5/nl). Anaemia Hb < 8mg/dl, thrombopenia < 100.000/mm3 =< 100/nl, panzytopenia, coagulation disturbances, eosinophilia > 1500/mm^3= > 1,5/nl absolute.

Kidney and bladder disturbances: Disturbances of the kidney function, severe micturation problems (urine retention, pollakisuria), incontinence,...

Sexual disturbances: Sexual disturbances which last > 4 weeks and are very bothersome for the patient, severe sexual disturbances, priapism,..

Endocrine and metabolic disturbances: Severe galactorrhoea, amenorrhoea > 6 months, symptomatic hypothyreosis, hyponatriemia <130 mmol/l; diabetes: new manifestation or exacerbation, hyperlipidemia which needs treatment, CK- increase > 2000 U/l, rhabdomyolysis.

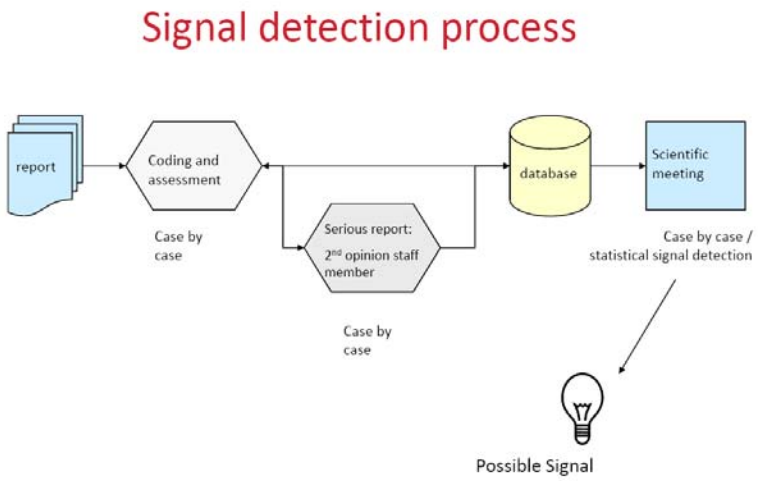
Respiratory disturbances: All forms of dyspnoea

Other ADR: Weight increase > 10% body weight, metabolic syndrome, binging attacks, severe lasting cephalea, fever > 39°C. Loss of efficacy will only be taken into account as consequence of a drug interaction.

Case and causality assessment are re-discussed in regional AMSP conferences and some complex cases in the international AMSP conferences as well. For estimation of the ADR frequencies, all medication from each surveyed patient is noted on 2 index days per year.

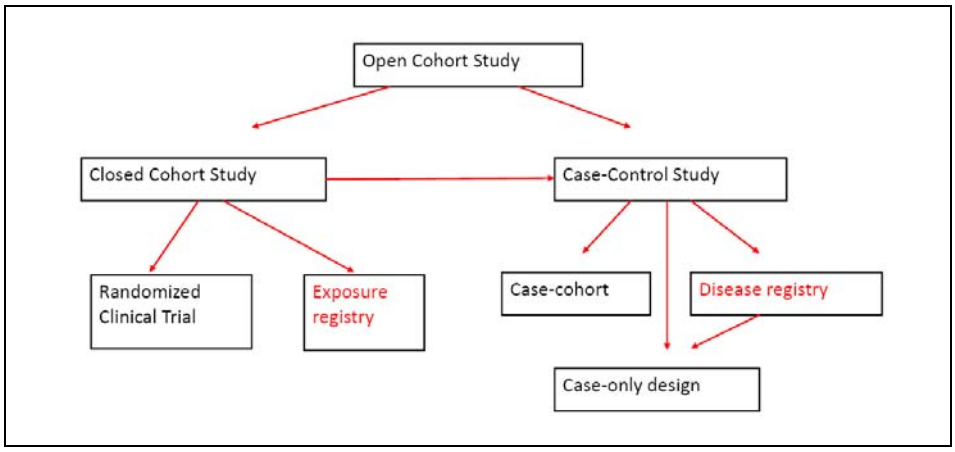
The AMSP project is a prospective multicentre dynamic cohort study under naturalistic conditions. Its aim is among others signal detection, in a case-by-case or qualitative analysis (18;19) as shown in figure 1. The signal can be a new ADR or a frequency change of an ADR associated to a certain drug or a change in severity.

Figure 1: process of signal detection



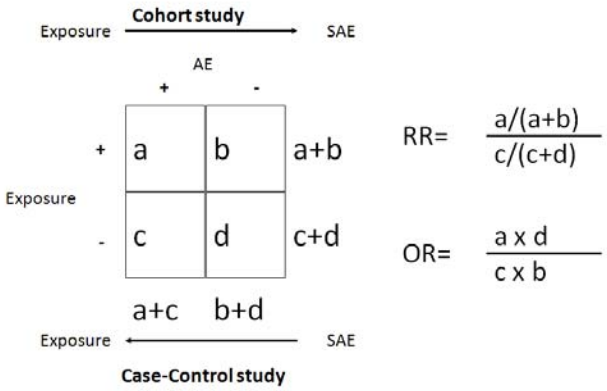
When a signal has been identified, a hypothesis is made which has to be verified in e.g. a case-cohort, case-control or other adequate epidemiological study (fig. 2).

Figure 2: Potential Study designs in epidemiology.



Epidemiological studies allow calculating incidences, prevalence and risk ratios; risk factors such as age, certain genotypes, drug interactions or certain diseases can be identified. For risk calculations, incidence in the exposed population versus incidence in the non-exposed population is compared (table 3).

Table 3: Two by two table for the calculation of risk ratios. (RR = relative risk (for cohort studies); OR = odds ratio (for case-control studies); SAE = serious adverse event; Exposure: exposed to e.g. a drug; a = exposed and SAE, b = exposed no SAE, c = non exposed, but SAE, d = non exposed and no SAE.)



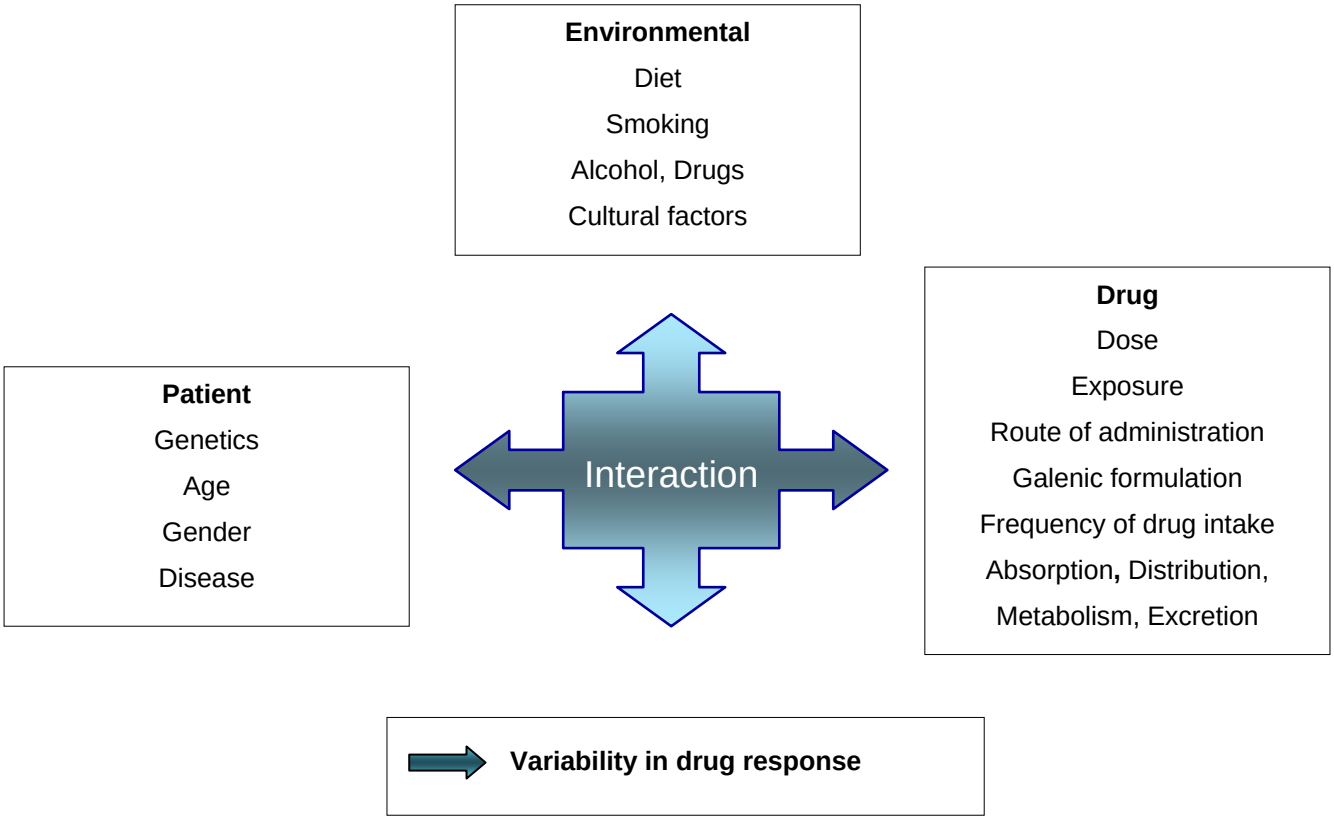
For more detailed methodology of the AMSP project see www.amsp.ch and (16;20). Some case reports from the AMSP project are presented in Chapter 8, a case-control study from a sub-population of the AMSP project in Chapter 6.

Inter- and intra-individual variations in drug plasma levels

Drug plasma levels are determined by pharmacokinetic parameters (absorption, distribution, metabolism, elimination = ADME) that determine the amount of drug reaching the site of action. Drug transporting proteins (e.g. P-glycoprotein (Pgp)) and most important drug metabolising enzymes (e.g. Cytochrome P450 (CYP450)) are relevant factors determining the pharmacokinetic profile. Drugs may be metabolised by many different sequential and/or competitive chemical processes comprising phase I metabolic reactions (oxidation e.g. CYP450, reduction, hydrolysis) and/or phase II reactions (e.g. glucuronidation, acetylation).

Inter- and intra-individual variations in drug plasma levels depend on biological variables and also on lifestyle and environmental factors. Figure 3 illustrates examples of factors influencing plasma levels and subsequently the risk of developing adverse drug reactions.

Figure 3: Interaction of drug, patient and environmental factors



Therapeutic Drug Monitoring TDM

TDM is based on the hypothesis that the concentration of a drug in the blood (plasma or serum) reflects - better than its dose - its concentration at target site. TDM is also based on the assumption that there is a definable relationship between drug plasma concentration and clinical effects (therapeutic effect and toxicity). In case of active metabolites the sum of the parent compound and the active metabolites (i.e. the active moiety) should be measured (e.g. venlafaxine and O-desmethylvenlafaxine ODV). This yields information on its contribution to the overall clinical activity of the compound, but also on the metabolism of the drug (e.g. ratio metabolite/parent compound).

In psychiatry, these relationships have been mainly investigated for lithium, tricyclic antidepressants and antipsychotic drugs (the latter with inconsistent results) (21-31). Methodological limitations of many studies might be the reason for the lack of an evident relationship between concentration and effects or side effects (32-36). However, systematic reviews and meta-analyses (37) based on adequately designed studies have produced convincing evidence for this relation. A correlation between plasma levels, dopamine D2 receptor occupancy and extrapyramidal side effects could be demonstrated for antipsychotic medication, e.g. for haloperidol (38;39). For drugs with a wide therapeutic index such as SSRIs, TDM is mainly used as a basis to adapt doses for special populations

such as the elderly, patients with hepatic impairment or patients with a known pharmacogenetic polymorphism affecting the metabolism of the prescribed drug (40).

Indications

Table 4 outlines indications where TDM is useful in relation to drug safety. In psychiatry, monitoring of substances with a narrow therapeutic index, especially when used in long term treatment and compliance control are probably dominant; TDM in particularly vulnerable patient populations may prevail in other medical specialities. TDM gains importance in the presence of unexpected adverse drug reactions.

Table 4: List of indications for TDM in relation with pharmacovigilance

o In case of adverse drug reaction type A
o Monitoring of substances with a narrow therapeutic window
o Combination therapy with pharmacokinetic drug-drug interaction potential
o Known pharmacogenetic polymorphisms (drug metabolic enzymes, transporter proteins)
o Pharmacotherapy in special patient populations (elderly, children, pregnant women, patients with renal or hepatic insufficiency)
o Problems occurring after switching different preparations of the same compound (e.g. original preparation versus generic)

Consensus guidelines for the use of TDM

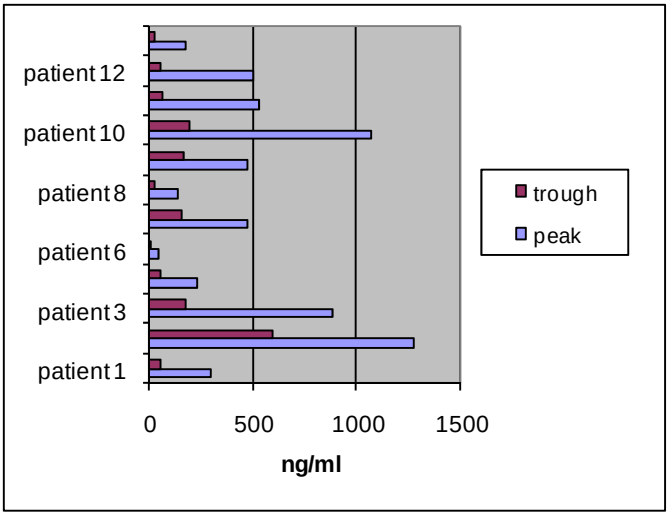
In 2004, the first international Consensus Guidelines for TDM of psychopharmacological agents were published by the interdisciplinary TDM expert group of the Arbeitsgemeinschaft für Neuropsychopharmakologie und Pharmakopsychiatrie (AGNP) (41), an update will be published in the fall of 2011. These guidelines cover indications for TDM, levels of recommendation, practical guidelines for clinicians and laboratories, and importantly, give reference plasma levels for the therapeutic window, as well as expected dose-dependent plasma levels under steady state conditions. Where therapeutic ranges are missing, target ranges corresponding to the normally observed plasma levels at therapeutic drug doses are given.

Sometimes, individual optimal serum concentrations seem to be preferred over consensus values, especially for long term and combination treatments (42;43). This is supported by studies on predicting relapse or rehospitalisation of patients under clozapine treatment; it was found that the variability in plasma levels in an individual patient seems to be predictive for a psychotic exacerbation (44;45).

In the difficult situation of a relative overdose (e.g. a very high plasma concentration due to a genetic deficiency for a metabolic enzyme or due to a drug-drug interaction (DDI)), it is important to know the toxic plasma levels of a drug. However, these are unknown for many, especially newer drugs, which in general have wider therapeutic indexes. Some listings of toxic drug concentrations exist (46;47); they have been generated by reviewing case reports of intoxications. A laboratory alert level has been included in the consensus guidelines 2011 indicating potentially harmful drug concentrations.

Reference plasma levels are generally based on trough steady-state concentrations. The difference between peak and trough levels can be very important, as e.g. in the case of quetiapine (fig. 4). Special attention is necessary when comparing results from immediate release galenic forms and extended release forms (48). Methylphenidate (49;50), atomoxetine (51;52) and agomelatine (CHMP assessment report of Valdoxan Procedure No. EMEA/H/C/000915) are exceptions, such that steady state peak plasma levels have to be measured. Methylphenidate and agomelatine have a very short half life, so inter-individual variability in drug pharmacokinetics and trough plasma concentrations are difficult to detect. In the case of atomoxetine the peak compared to trough plasma concentrations are less affected by the CYP2D6 genotype-dependent inter-individual variability. In patients treated with an intramuscular depot preparation of an antipsychotic drug, blood should be sampled immediately before the next injection and also during steady state conditions (often only reached after 2 - 4 months) (53).

Figure 4: peak (blood sampling 90 minutes after drug intake) and trough plasma concentrations of quetiapine immediate release of 13 patients participating in a multicentre pharmacogenetics study on quetiapine (manuscript in preparation)



TDM is a valid tool to optimise pharmacotherapy, but it does not replace clinical judgement. Since the majority of adverse drug reactions is dose-dependent, measuring drug plasma levels seems to be a highly rational approach to prevent these reactions, to reveal possible causes and, subsequently, to take steps to adjust drug treatment.

Pharmacogenetics

Definition

Pharmacogenetics describes hereditary factors influencing the response to drug treatment - therapeutic effect and potential side effects - either dealing with the fate of drugs in the body (ADME) or the interaction of the drug with the body at the target site (pharmacodynamics, e.g. neurotransmitter transporter polymorphism). “Genetic polymorphism” refers usually to genetic loci for which variants occur with a frequency of at least 1% (54).

Cytochrome P450 polymorphisms

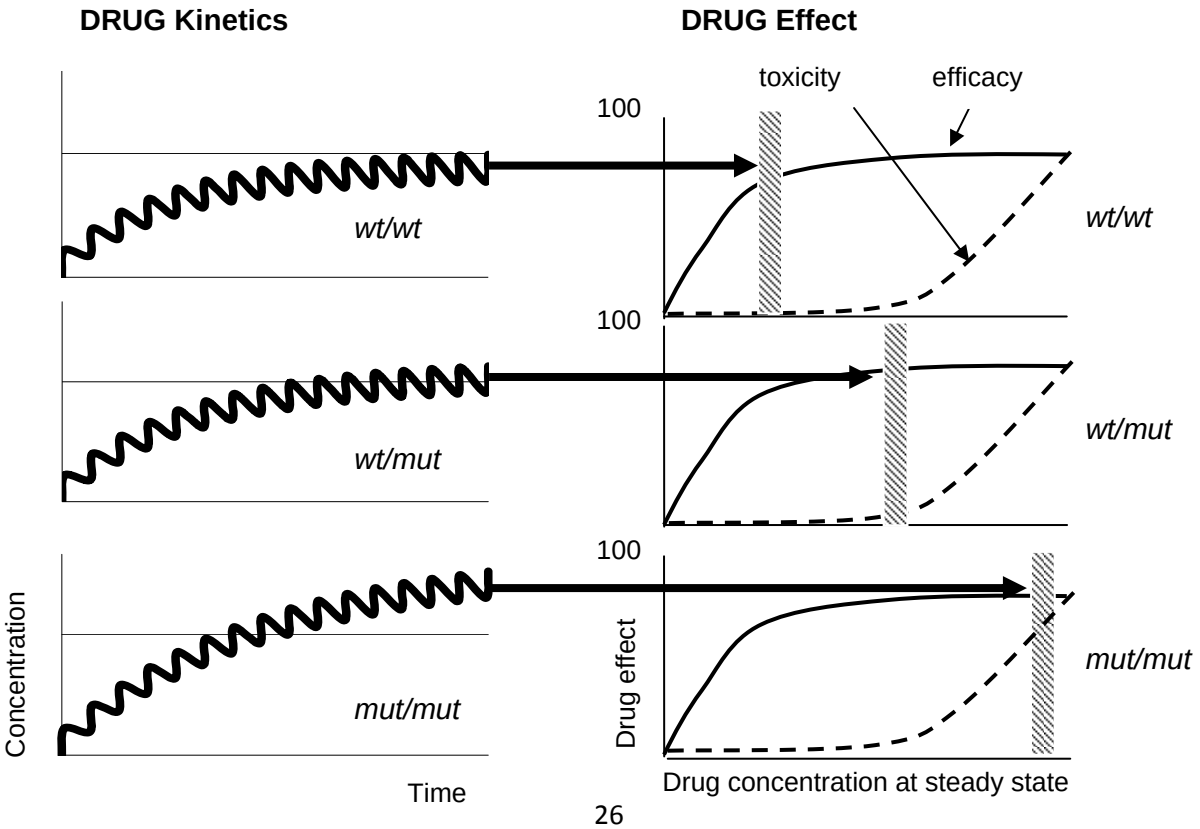
Genetic polymorphisms of drug metabolising enzymes and their effects on treatment response for certain patients have been extensively studied since the late 1970s (55-57). Individual genetic disposition determines their activity, and the number of active alleles in a gene determines to a great extent how much enzyme will be produced. Some Cytochrome P450 isozymes are primarily involved in phase I reactions of psychoactive drugs, most importantly CYP2D6, but also CYP1A2, CYP2B6, CYP2C19 and CYP3A. CY2D6 and CYP2C19 are genetically polymorphic, some genotypes leading to complete enzyme deficiency. CYP3A as well as CYP1A2 are largely influenced in activity by enzyme induction or inhibition but genetic polymorphisms in these two CYP enzymes are less important for the phenotype. From a genetic point of view, four types of drug metabolisers have been identified, which are not found with all drug metabolising CYP450 enzymes. The most comprehensively studied CYP isoform is CYP2D6 and the following definitions are established mainly on the basis of observations about the metabolism of substrates by this enzyme.

1. ”Poor metaboliser” (PM) carry two alleles predicting a low (e.g. CYP2D6*10 or *17)(58) or no enzyme activity (e.g. CYP2D6*3, *4, *5);
2. “intermediate metaboliser” (IM) are normally referred to individuals being heterozygous carriers of one inactive allele or have two alleles with reduced activity, leading to a reduced enzyme activity;
3. ”extensive metaboliser” (EM) are carriers of two active alleles having a normal activity;

4. ”ultra-rapid metaboliser” (UM) have a very high enzyme activity which is genetically caused by gene duplication expressed as 2XN (so far found for CYP2D6). Recently rapid metabolisers for other CYP450 enzymes have been found, e.g. in patients carrying the CYP2C19*17 allele (59;60) .

The phenotypes reflecting the actual enzyme activity still show high inter-individual variation especially within the intermediate and extensive metaboliser groups. Thus, genetic prediction of enzyme activity is best possible for the poor and ultra-rapid genotypes but poor or ultra-rapid metabolizing activity can also be caused by enzyme inhibition or induction (61;62). Instead of this classical approach of attributing phenotypes to genotypes, efforts have been made to predict a CYP450 enzyme activity score by genotype (63). Figure 5 illustrates genotype dependent plasma concentrations and expected therapeutic and toxic effects. Extensive information on the activity of different CYP450 alleles can be found on www.imm.ki.se/CYPalleles.

Figure 5: Schematic illustration of CYP2D6 genotype-based plasma concentration differences and therapeutic consequences (64) (wt = wild type, mut = mutant; ▨ = genotype based plasma concentration at steady state). In the case of a person with the wild type genotype there is an optimal balance between drug efficacy and toxicity, in the case of a person with homozygote mutant genotype the risk for toxicity is high.



The prevalence of different types of metabolisers varies greatly between ethnic groups (table 5) (65).

Table 5: Estimate of the prevalence of relevant CYPP450 polymorphisms in different ethnic populations

Ethnicity	**CYP450**	**Poor metaboliser**	**Ultra rapid metaboliser**
Various*	1A2*	Rare (66-68)	Induction polymorphism (68-72) Clinical relevance unclear
- Caucasian (73-75) - Asian (76;77) - African (78)	2C8/9	1-10% 0-2% up to 4%	None
- Caucasian (75;79), African (80), Saudi Arabia (81) and Turkish (82) - Asian (77;83-85)	2C19	1-5%, 13-23%	CYP2C19*17 (59;60)
Various	2B6	High inter-ethnic differences (86-92)	CYP2B6*4 (93)
- Asian (79;94) - Turkish (82) - African (80;94) and Afro American - Caucasian (75;95-99) -Saudi Arabia (81;100) - Aethiopian - Asian (101) - African (80)	2D6	1-2% . 2-4 % . 5-7% Carrier of an allele with reduced activity(102-104) up to 50% (CYP2D6*10) up to 30 % (CYP2D6*17)	up to 2% 5-10% 2% . 1-2 % North Europe 5-10% South Europe 20% up to 29%
- Caucasian (105) - Afro Americans (105) - Japanese (106) - Chinese (107)	3A5	About 70 % About 40% 30-40% about 50%	
Various (108)	3A**	Wide variability in metabolic capacity, few functional polymorphisms identified.	Wide variability in metabolic capacity, few functional polymorphisms identified.

* The inter-individual variability of the CYP1A2 metabolic capacity is wide, with a bi- or tri-modal distribution depending on the population; only few functional genes have been identified to date.

**The inter-individual variability in the CYP3A metabolic capacity is wide but no bi- or multimodal distribution has been found, indicating that most probably several genes contribute to the function.

CYP2D6 and CYP2C19 show a polymodal distribution of activity in the population. For CYP2D6 which catalyses the oxidative biotransformation of many tricyclic antidepressants and other psychotropic drugs, 5 to 8% poor metabolisers and 1 to 10 % ultra-rapid metabolisers have been found in Caucasians. CYP2C19 polymorphisms are less prevalent in Caucasians and seem to be therefore less important (109) although several tricyclic antidepressants and citalopram are catalysed by this enzyme. In Asians, however, about 20% of the population are poor metabolisers. The unimodal distribution of large inter-individual variability of CYP3A4 activity suggests multiple influence factors on enzyme activity of this important enzyme which is highly expressed in human liver. Two other CYP3A enzymes are existing, CYP3A5 for which genetic polymorphisms have been detected to predict enzyme expression, and CYP3A7 which is mainly expressed during foetal life and later in a low percentage of adults (110;111). CYP3A5 is only expressed in 10-30% of the Caucasians and their contributing effect to overall CYP3A activity is low. The clinical relevance of the CYP1A2 polymorphism (C->A) (66;70) associated with high inducibility is unclear and conflicting results have been reported (69;71;112). Genetic and environmental factors can interact synergistically or in an antagonistic way by e.g. adding a CYP2D6-blocking agent in pharmacological treatment with a CYP2D6 substrate taken by a CYP2D6 ultra-rapid metaboliser (113).

Recent investigations indicate that drug transporters, such as P-glycoprotein (Pgp) and organic anion-transporting polypeptides (OATP), in the intestinal mucosa and the blood brain barrier are also relevant for the pharmacokinetic variability of many drugs (114-119).

P-glycoprotein, coded by the MDR-1 (multi-drug resistance, also known as ABCB1) gene, is an adenosine triphosphate (ATP)-dependent efflux pump for xenobiotic compounds with broad substrate specificity (120;121). A model of Pgp-mediated substrate transport (fig. 6) can be found in recent publications (122). Pgp plays an important role in drug absorption, disposition and excretion, and is found in several organs such as the gut, liver, gonads, kidneys, brain and others (116;117;123;124) (fig. 7). Reported genetic polymorphisms of MDR-1 show high inter-ethnic variability and appear to play a role similar to that of drug-metabolizing enzymes (114;125-129). Pgp function can be influenced by drugs, food, smoking, age and gender, and it can be inhibited or stimulated. Interestingly, Pgp and CYP3A4 are often co-expressed in the same cells and they share a large number of substrates and modulators (inhibitor and inducer). The disposition of such drugs is influenced by both drug transport and metabolism, and the interaction with modulators acting on both systems will multiply the effect; for example, cyclosporine is a substrate of CYP3A4 and Pgp (130-132), whilst St. John’s wort is an inducer of both (133-135).

Figure 6: Model of substrate transport by the efflux pump Pgp (lilac), membrane between two horizontal lines. Pgp-substrates (pink) enter a cavity of the P-glycoprotein lined up with amino acids (blue) which can bind to many different molecules. ATP (adenosine triphosphate) binds to two nucleotide-binding domains which causes a conformational change ejecting the substrate to the outside. Adapted from (122)

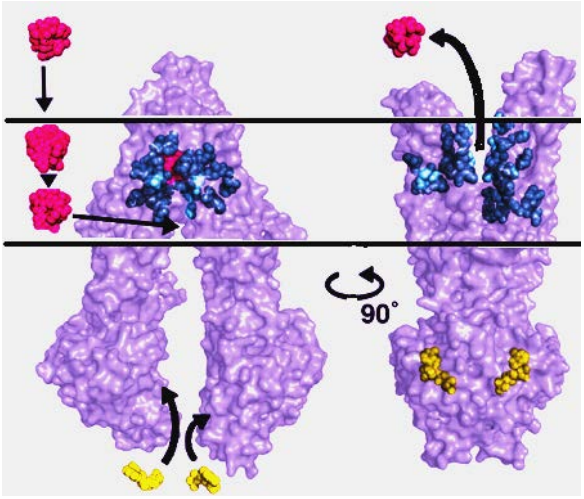
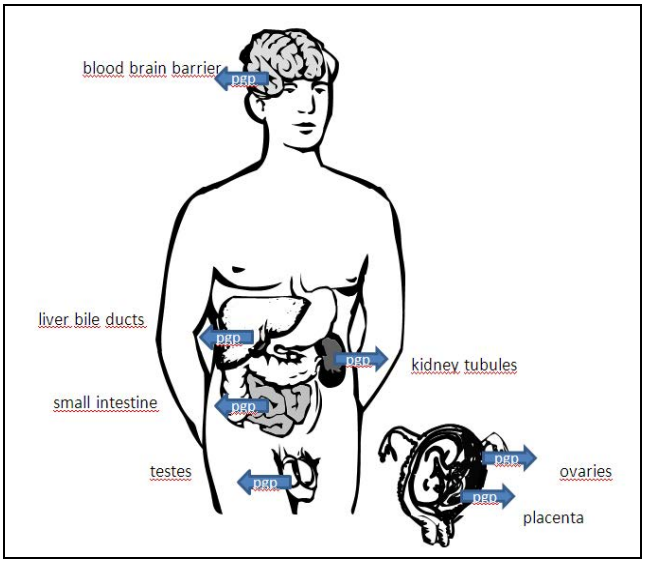


Figure 7: Pgp acts as an efflux pump for xenobiotics at the brain, the liver, the intestine, the gonads, the kidney, and at the placenta in order to protect vital organs. Adapted from (136)



With regard to the occurrence of wanted or unwanted clinical effects, the contribution of drug transporters is less well understood than that of drug metabolising enzymes. Digoxin is a typical Pgp substrate with a narrow therapeutic index that can be affected by Pgp inhibition or induction. Certain

phenotypes of Pgp can lead to increased plasma levels of digoxin (125) and subsequently to serious adverse drug reactions. Another example is the Pgp substrate loperamide, a potent opiate anti-diarrhoea drug that has limited access to the brain due to Pgp activity. When combined with the Pgp inhibitor quinidine, it can enter the brain and cause respiratory depression (137) without any change in plasma levels of loperamide.

Pharmacogenetic tests

Depending on the particular CYP450 enzyme, different geno- or phenotyping methods are today available for the clinician. Genotyping and phenotyping differ in their clinical significance. Genotyping is considered as a "trait marker" and its result does not depend on environmental factors, meaning that it has only to be performed once in a person's lifetime. In general a DNA probe is extracted from a non-centrifuged whole blood sample, but buccal swabs or saliva samples may also serve. Most laboratories analyse only the most common alleles for which a functional significance is known. Standard methods such as real-time polymerase chain reaction (PCR) (138) are mainly used for rapid and inexpensive genotyping of the common alleles. More than 95% prediction of the poor metaboliser of CYP2D6 is possible with genotyping of the (few) alleles predicting deficient enzyme activity such as CYP2D6*3, *4, *6 and *5 (104;139-141), and for CYP2C19 80% and almost 100% of the poor metaboliser can be predicted in analysing CYP2C19*2 and *3 (142) in Caucasian and Asian respectively. In cases where many alleles of more than one gene should be analysed, microarray-based genotyping devices ("gene chips") (143;144) are recommended. Unfortunately the costs for gene chip analysis are still relatively high. A very short time lag between collecting a DNA probe and obtaining the results is a prerequisite if genotype-based dosing is applied.

Phenotyping tests exist for more CYP450 enzymes but they represent "state markers" meaning that they are situation dependent. This carries the advantage of reflecting the metabolic situation of the patient at a specific moment, and allows its evolution to be followed. Some persons especially among psychiatric patients experience the fact that they have to ingest a test substance which will be later analysed in a blood or urine sample together with its metabolites as disadvantage. Phenotyping test probes should be isozyme specific such as dextromethorphan (145), sparteine/desbrisoquine for CYP2D6 (146;147), mephenytoin (145) or omeprazole for CYP2C19 (148), tolbutamide or flurbiprofen for CYP2C9 (149;150), caffeine for CYP1A2 (69) and midazolam for CYP3A (151;152). However, some of these probes lack enough specificity as is the case for dextromethorphan to clearly distinguish ultra-rapid from extensive metaboliser (and intermediate metabolisers).

Genetic association - haplotype analysis (153)

Single nucleotide polymorphisms (SNPs) in a gene can be analysed either considering them each as a separate predictor or by haplotypes. A haplotype is a combination of alleles or SNP's, a set of DNA variations, located on the same chromosome and which tend to be inherited together. Special statistical methods exist for inferring haplotypes and population haplotype frequencies from the genotypes of unrelated individuals. These methods, and the software that implements them, rely on the fact that in region of low recombination relatively few of the possible haplotypes will actually be observed in any population. True haplotypes are more informative than genotypes, but inferred haplotypes are typically less informative because of uncertain phasing. However, the information loss that arises from phasing is small when linkage disequilibrium is strong *(153)*.

These complex statistical methods are commonly applied when studying the association of ABCB1 SNPs to e.g. drug plasma concentrations or drug effects (see e.g. chapter 5).

Pharmacogenetic Studies in Pharmacovigilance

Sufficient clinical data are missing to give clear cut recommendations concerning pharmacogenetic testing before initiating treatment. Kirchheiner et al (154-156) developed a scheme for genotype-based dose adjustments. They recommend genotyping for drugs where a minimum 2 fold difference in AUC for the active moiety has been observed between poor metabolisers and ultra-rapid or extensive metabolisers and/or for which a twofold or more risk for an adverse drug reaction or therapy failure exists. Some clinical studies in psychiatry found a higher number of patients not tolerating treatment as a consequence of a genetically deficient drug metabolism (157-164). In case of genotype-based dose adjustments the drug plasma concentrations will be controlled by TDM since most genotype-based dose recommendations are based on calculations rather than on clinical data.

TDM and pharmacogenetic tests can advantageously be combined, and TDM can to a certain extent be considered as a phenotyping procedure. A valuable strategy is proposed in the algorithm in chapter 6 "TDM plus" (65). It reflects a pro-active and systematic approach to a situation of treatment failure or intolerability where pharmacogenetic tests are performed based on unexpected plasma levels and after exclusion of pharmacokinetic drug interactions. Pharmacogenetic tests might also be indicated in the case of unusual plasma concentration to dose relations or when the ratio of parent substance to metabolite is distorted.

Polymorphic drug metabolising enzymes represent some of the most common genetic risk factors associated with adverse drug reactions (165-169) but may also be the reason for non-response. In poor metabolisers increased plasma concentrations can reach toxic levels and lead to serious adverse drug

reactions, in ultra-rapid metabolisers on the contrary, non-response might occur due to subtherapeutic plasma concentrations. Differences in drug clearance between poor and rapid metabolisers sometimes vary up to 20-fold which certainly has an influence on drug efficacy and adverse drug effects. However, if analyzing phenotypes such as drug responders or occurrence of adverse drug effects, a simple monogenic association between a single polymorphic drug metabolising enzyme and response or an adverse event, seems in many cases not evident. Therefore new high-dimensionality analysing methods like "combinatorial pharmacogenetics" (170) which allow an insight in the complexity of possible metabolic processes and pathways in the human body are necessary. Other for the pharmacokinetic variability of many drugs relevant systems such as phase II enzymes (e.g. UDP-glucuronosyltransferases UGT), N-acetyltransferases (NAT)) and drug-transporting-proteins, e.g. P-glycoprotein and organic anion-transporting polypeptides (OATP), show genetic polymorphisms as well. The clinical relevance of phase II enzyme polymorphisms such as UGT polymorphisms in pharmacopsychiatry seems to be far less pronounced than those of CYP450 isozymes (171). Genetic polymorphisms of the drug transporter Pgp have been studied extensively, but the functional significance of genotypes or haplotypes remains controversial (136;172).

No doubt, pharmacodynamic parameters are important and clinically relevant genetic polymorphisms for receptor proteins or neurotransmitter transporters have been described (173-176). For instance numerous studies show a robust association between the serotonin transporter gene promoter polymorphism and the therapeutic effect of SSRI (177). Therefore, results of pharmacogenetic tests for pharmacokinetic and –dynamic variables should - where possible - be analysed together. However, the use of pharmacogenetic tests for pharmacodynamic parameters is not yet validated in clinical practice.

Drug-Drug Interactions

Drug-drug interaction means a change of the drug's effect as a result of the presence of another drug. Considering pharmacokinetic and pharmacodynamic drug interactions including the risk increase for serious adverse reactions due to similar side effect profile, the risk estimation of a certain drug combination can become very complex. Classical information sources on drug interactions such as Pharmavista (http://www.pharmavista.net) or Drugdex (http://www.thomsonhc.com) give risk estimations based on clinical observations, and deducted from these class effects. However, this method is often not accurate and incomplete. Another more precise approach would on one hand include – if available - clinical observations of a specific drug combination and on the other hand look at the pharmacokinetic and pharmacodynamic properties of the combined drugs as well as at their side effect profile and make then risk estimation.

Pharmacokinetic interactions

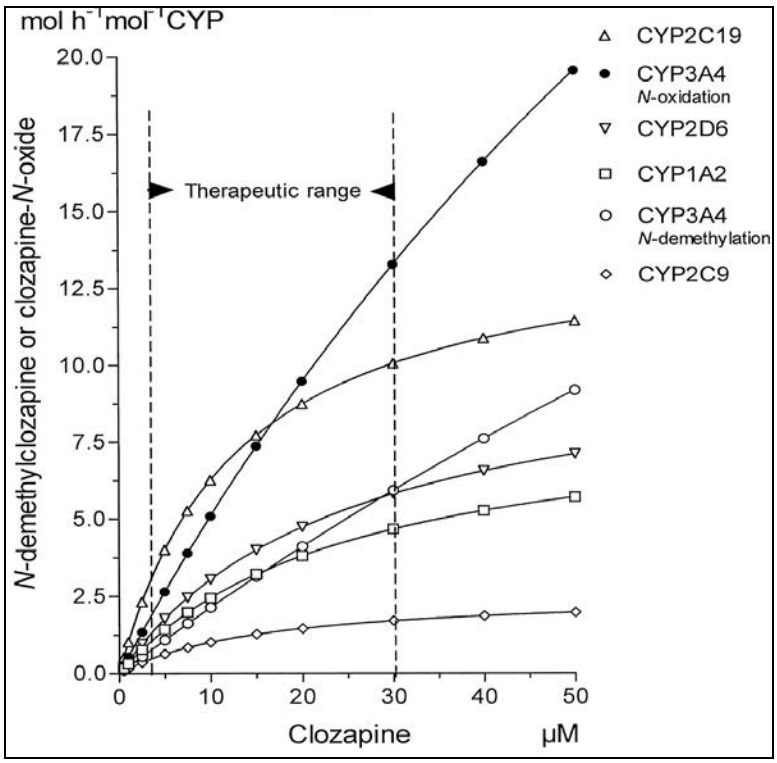
Pharmacokinetic interactions are due to the effect of drug A on drug B's movement through the body. Alterations can occur during absorption, distribution, metabolism and elimination. They are expressed by a change in the expected concentration of one or both substances at the target site, and often also in the blood. TDM is therefore a valuable instrument in controlling the effect of a pharmacokinetic drug interaction, even if it is not a direct measure of the drug concentration at the target site. The usefulness of TDM may be limited in situations where drug transport through the blood brain barrier shows high inter-individual variability and is determined by active transport mechanisms (116;123). Information on mechanisms of metabolic interactions can be found in text books (178) or other literature (179;180).

Drugs can be substrates for one or several metabolic enzymes, which contribute to their biotransformation using major and minor pathways (e.g. clozapine (181), see Fig. 8) and drug metabolism can be stereoisomer specific (e.g. mirtazapine, see chapter 4) . This is important when estimating the effects of inhibition or induction of one of these pathways. The extent of an interaction is dependent on the baseline enzyme activity. No inhibition occurs in people with almost no enzyme activity (e.g. in the situation of a genetic deficiency of this enzyme), whilst the inhibitory effect may be pronounced in people with high baseline activity.

It is not an easy task to estimate the interaction potential of a particular combination therapy. Numerous tables listing drugs as substrates and inhibitors/inducers for different metabolic enzymes, mostly CYP450 enzymes exist. Many do not differentiate between major and minor pathways, and many translate in vitro results into in vivo data, which can lead to misinterpretation. Clozapine in vitro is metabolised by almost all relevant CYP450 enzymes (fig.8 (181)). However, in vivo (see chapter 5), it appears that CYP1A2 is the major pathway, CYP2C19 plays a relevant role (182) and CYP3A4 is probably involved in a concentration-dependent manner, while CYP2D6 plays a negligible role (181;183-186).

Predicting in vivo interactions from in vitro data is difficult; a number of reviews have been published on the impact of various factors on the accuracy of such an extrapolation and on prediction models (187-191). There is great need for data on drug metabolism and transport *in vivo* and where possible in patient populations. With newer drugs this information becomes more accessible since more pharmacokinetic data are requested from the authorities. But with older substances these data are not available.

Figure 8: Dose dependent CYP450 mediated metabolism of clozapine in vitro (181), reaction rates in mol/h per mole CYP450 isoform. (Reproduction of the figure with kind permission of "Drug Metabolism and Disposition")



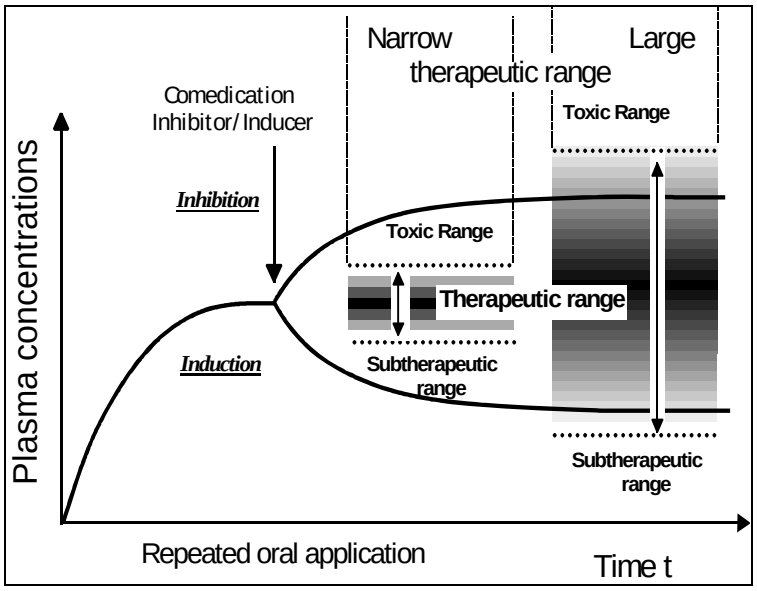
Some drug interaction lists give the interaction potential of drug classes, such as found for the SSRIs, but SSRIs form a very heterogeneous group especially concerning their CYP450 enzyme inhibiting properties. Fluvoxamine is a potent inhibitor of CYP1A2, but not of CYP2D6. Paroxetine and fluoxetine are potent inhibitors of CYP2D6, but not of CYP1A2, and so forth. The thesis author, recognising these shortcomings, developed an online interaction program, mediQ.ch, which is described in greater details in chapter 7. Table 6 indicates some Internet sites with clinically relevant information on drug interactions, cytochrome P450 and other drug metabolising and transporter systems.

Table 6: Examples of Internet sites providing information on drug-drug interactions, CYP450 enzymes and drug-transporting proteins (retrieved 2011)

www.mediQ.ch	www.psiac.de	http://medicine.iupui.edu/flockhart
www.genemedrx.com	www.imm.ki.se/CYPalleles	www.themedicalletter.com
www.drugs.com	www.druginteractioninfo.org	http://www.thomsonhc.com
http://www.pharmavista.net	http://www.hiv-druginteractions.org	

Since the extent of a specific drug-drug interaction is not easy to predict, TDM should be used in drug combinations where affected drugs have a narrow therapeutic index. Figure 9 shows that the clinical consequences strongly depend on the therapeutic index of the drug. It would not be wise to avoid combinations when they appear to be of little risk and promising from a therapeutic point of view. Inhibition can last for several weeks after discontinuation of the inhibiting agent, as is the case with fluoxetine, and especially its metabolite norfluoxetine, which has a very long elimination half-life. In cases of a rapid change of medication from fluoxetine to another serotonergic compound, an increased risk for serotonergic side effects, including serotonin syndrome (192-195) has been reported.

Figure 9: The importance of a drug-drug interaction or a drug metabolising enzyme polymorphism depends on the therapeutic index of the affected drug (adapted from (196))



For drugs with active metabolites, the active moiety has to be considered, especially when the active metabolite is formed by the affected enzyme. In a study with 12 schizophrenic patients (197) risperidone was shown to be inhibited by paroxetine in a dose-dependent manner. Daily doses of 10, 20 or 40 mg paroxetine resulted in a 3.8- to 9.7- fold increase in the concentration of risperidone. The concentration of the “active moiety” (risperidone + 9-OH-risperidone) was not significantly increased by low doses of paroxetine, but a 1.8-fold increase occurred after 40 mg/day paroxetine. However, extrapyramidal side effect scores increased significantly also with 20 mg/day paroxetine.

Modulation of drug metabolism can be enantioselective, as is the case for warfarin, methadone, some antidepressants (e.g. venlafaxine, citalopram, mirtazapine) and other substances (198-206). Since in

many cases the effect of each enantiomer is distinct, it is important to know which metabolic pathway is affected.

In addition, it should be noted that an inhibitory effect occurs as soon as the inhibitor is introduced and disappears – with exception of mechanism based inhibition - as soon as the interacting compound is eliminated from the body, which implies that the time course depends on the elimination half-lives of the drugs (and metabolites) implicated in the interaction.

The induction process takes time since more, new enzyme has to be synthesized. As a rule of the thumb, one could say the induction effect can generally be expected after one week and the full effect might take several weeks. This has to be kept in mind when applying TDM. When an inducer is removed from treatment (207), plasma levels of the substrate will increase with about the same lag time until a new equilibrium is reached.

Pharmacodynamic interactions

Pharmacodynamic interactions are due to the influence of drug A on drug B at the target site of drug action (end organ, receptor site). Serious complications such as serotonin syndrome, resulting from a combination of several serotonin agonistic drugs (e.g. SSRI plus the analgesic tramadol or the anorectic sibutramin) (208-210), or delirium caused by a combination of drugs with anticholinergic properties, are examples. Pharmacodynamic interactions are not easily measured in vivo.

Other Interactions

Individual drug response is also dependent on factors, such as age, gender, organ function (especially renal and hepatic), co-morbidity but also lifestyle or environmental factors like diet or smoking. These factors certainly affect CYP450 enzyme function but glucuronidation and the expression of drug transporters also seem to be sensitive.

Smoking

Smoking induces CYP1A2 which means that smokers are likely to have lower plasma levels of CYP1A2 substrates than non-smokers. Importantly, the tar particles in the smoke rather than nicotine are responsible for this effect. A similar effect is also seen when consuming barbecued meat, for instance. Smoking also slightly induces glucuronidation, as seen with codeine (211). Environmental and genetic factors can produce either synergistic or antagonistic effects. It appears that there is also a genetic polymorphism for inducibility (e.g. by tobacco smoke) of CYP1A2 (66;70).

Smoking cessation in patients on drugs like clozapine, olanzapine, tacrine or theophylline, which are mainly metabolised by CYP1A2, can lead to drug intoxication. Several cases have been described for

clozapine and olanzapine, (212-214) with adverse drug reactions including seizures, heavy sedation, cardiac problems and delirium. The induction effect of smoking seems to have a mean elimination half life of about 39 hours (range 27 - 54h) (207); a new steady state could be expected after about 2 weeks. De Leon in his study overview (213) suggests a mean dose correction factor of 1.5 for change in smoking behaviour. In individual patients, however, smoking cessation may lead to a more marked increase in plasma levels. Other authors refer to mean correction factors of up to 5 (71;215). A stepwise dose reduction with TDM control is strongly recommended.

Food

Recently there has been increased awareness that grapefruit juice can have an important interaction with as many as 40 orally taken drugs (216;217). In particular, interaction with certain HMG-CoA reductase inhibitors (statins), such as simvastatin, atorvastin and lovastatin, can lead to serious complications such as rhabdomyolysis (217;218); with some antihypertensive agents (e.g. felodipine or nifedipine) it might result in excessive vasodilatation. For drugs with a narrow therapeutic index such as the immunosuppressant cyclosporine or the antimalarial agent halofantrine special attention is necessary. In psychiatry, drugs such as midazolam, triazolam, buspirone, carbamazepine or quetiapine are affected. The type of interaction consists mostly in an increase in drug plasma levels, seen in either the area under the concentration-time curve (AUC) or the maximum plasma concentration (C_{max}). The main mechanism is the inhibition of the intestinal CYP3A4 pathway, inhibition of P-glycoprotein might play a role as well. A review on pharmacokinetic interactions with citrus juices (mainly grapefruit) (216) gives more insight in possible mechanisms of action.

Interactions with other food constituents (caffeine, cabbage, chargrilled food, water cress, and others) exist but, with the exception of the combination of clozapine and caffeine (219;220), seem to play a less important role.

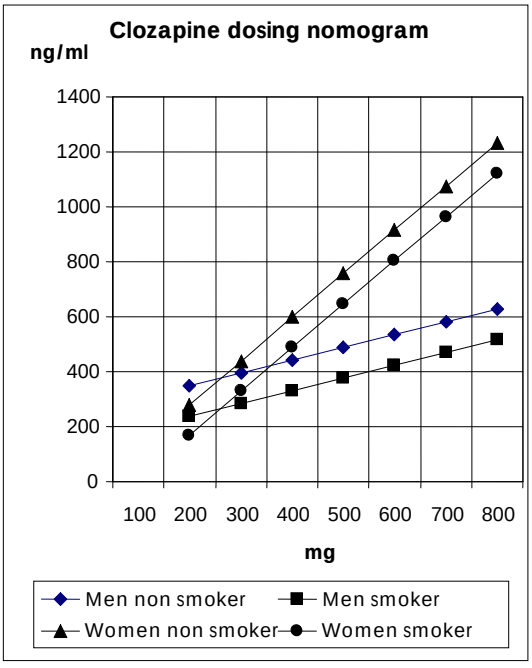
Age and gender

Divergent responses to drug treatment are often observed between the elderly, young and adult population. In children, some clinical studies indicate that higher doses (on a weight-adjusted basis) are often needed, as compared with adults, to reach therapeutic drug concentrations (221;222). This seems to be based on an increased clearance of the drug in younger children. In contrast, in elderly patients, impaired renal and sometimes hepatic function often leads to a decrease in drug elimination and reduced drug metabolism, respectively, and therefore dose adaptation may be recommended (196;197). It is also probable that the therapeutic index of some drugs in some individuals is narrower, due to an increased sensitivity to drugs in the elderly (223).

Men and women often differ in their response to drug treatment. Gender differences in subjective tolerability might account for part of this (224), but biological factors are certainly also important (225). An extensive overview of gender-specific factors such as body build, hormonal transitions, diet and other environmental or cultural factors was recently presented in relationship to antipsychotic therapy (226). The author states that, for a given dose, the mean plasma levels in men tend to be lower than in women and concludes that the evidence collected suggests that women need lower doses than men. Another review (227) mentions that the adverse drug reaction risk for women is about 1.5-fold greater compared to men. Own data from a naturalistic cohort study including 165 psychiatric in-patients with a severe adverse drug reaction showed that women (n=79) were more likely to have unexpectedly high plasma drug levels than men (n=82) (36% versus 22% (228). This gender difference has been demonstrated previously and the findings have been reviewed by Pollock et al (229). The use of TDM rather than adoption of a standard recommended dose could help optimise individual doses of therapeutic agents.

Pronounced gender differences are, for example, described for clozapine (230;231) and olanzapine (232), both of which are mainly metabolised by CYP1A2. Perry et al (230) developed a clozapine dosing model comprising the variables dose, smoking and gender. To reach therapeutic plasma levels, smoking men may need twice the dose required by non-smoking women (fig. 10).

Figure 10: Gender-related dose differences for clozapine (adapted from (230))



Gender differences are observed in drug metabolizing enzymes and drug transporter proteins (233;234). Men appear to have higher CYP1A2 activity, and maybe also CYP2E1, as well as of some UGTs and Pgp, while women may have higher CYP2D6 activity (235). Of course there are other physiological differences between women and men: women have generally lower bodyweight and organ size, a higher percentage of body fat, lower glomerular filtration rate and different gastric motility than men.

In general, however, gender-based pharmacokinetic differences account for only subtle changes in drug response; gender-based pharmacodynamic processes such as QTc prolongation seem to be more important (234).

Co-morbidity

The effect of renal or hepatic insufficiency on the fate of a drug may be dramatic, but it depends on the means of elimination. Not widely known is that plasma levels of CYP1A2 substrates can vary in the presence of an inflammatory process and lead to an intoxication with drugs such as clozapine. The hypothesis is that cytokines (e.g. interleukin-6) inhibit CYP1A2 activity (236). Several animal studies have shown that different CYP450 enzymes are down-regulated during sepsis (237) or after endotoxin-induced inflammation, but the mechanism for this reduction is still under debate.

In this introductory chapter, present knowledge is summarized about the basics of pharmacovigilance, TDM and pharmacogenetics, as well as of drug-drug interactions and other factors influencing the drug concentration in our patient's body.

4 Multicentre study on the clinical effectiveness, pharmacokinetics and pharmacogenetics of mirtazapine in depression

Summary

*Pharmacogenetic tests and therapeutic drug monitoring may considerably improve the pharmacotherapy of depression. The aim of this study was to evaluate the relationship between the efficacy of mirtazapine (MIR) and the steady-state plasma concentrations of its enantiomers and metabolites in moderately to severely depressed patients, taking their pharmacogenetic status into account. In- and out-patients with major depressive episode (17-item Hamilton Depression Rating Scale (HAMD) total score ≥18 points and Mini-Mental State Examination score ≥24) received MIR for 8 weeks (30 mg/day on days 1-14 and 30-45 mg/day on days 15-56). A total of 45 patients (mean age 51 years; range 19-79) were included. MIR treatment resulted in a highly significant ($p < 0.0001$, Wilcoxon test) improvement in mean HAMD total score at the end of the study. The analysis of the enantiomers of MIR and its hydroxylated (OH-MIR) and demethylated (DMIR) metabolites in non-hydrolysed and hydrolysed plasma samples on days 14 and 56 showed a clear influence of gender and age on these parameters. Moreover, non-smokers had higher MIR plasma levels than smokers: S-MIR: 9.40±3.85 vs. 6.15±5.50 ($p = 0.005$); R-MIR: 24.4±6.54 vs. 18.5±4.06 ($p = 0.003$). Only in non-smokers, plasma levels of S-MIR and metabolites depended on the CYP2D6 genotype. In patients presenting the CYP2B6 *6/*6 genotype (n = 8), S-OH-MIR concentrations were higher than in the other patients (n = 37), and the reduction of the HAMD scores was significantly more pronounced in the CYP2B6 *6/*6 genotyped patients at the end of the study. However, it is not known, if S-OH-MIR is associated to the therapeutic effect of mirtazapine.*

Introduction

Pharmacogenetic tests and therapeutic drug monitoring of psychotropic drugs are increasingly recommended for the optimization of the pharmacological treatment of depression (41;64;154;238). Many antidepressants are chiral drugs in that they possess one or several asymmetric centres which give rise to enantiomers differing by their metabolism, pharmacokinetics and pharmacological properties. However, the majority of studies on the drug plasma concentration - clinical effectiveness relationship of chiral antidepressants were usually carried out using achiral analytical methods, which are unsuitable for the assay of the individual enantiomers (199;239).

Many studies have documented the clinical effectiveness of the chiral antidepressant drug mirtazapine (MIR) in the treatment of depression (240), including in elderly patients (241;242). It has an unusual pharmacological profile (table 7) (243), acting as an antagonist at central presynaptic α_2-adrenergic

inhibitory autoreceptors and heteroreceptors, thereby causing an increase in the release of noradrenaline. The subsequent excitation of postsynaptic α_1-receptors, which mediate serotonin (5-HT) cell firing, and the direct blockade of inhibiting α_2-heteroreceptors located on 5-HT terminals, possibly lead to an increase in the release of 5-HT. The effect of the released 5-HT is exerted mainly via 5-HT_1 receptors, since 5-HT_2 and 5-HT_3 receptors are blocked directly by the drug (244-246). The extent of the serotonergic effect of MIR is, however, somewhat controversial (243;247;248). The S(+) enantiomer of MIR (S-MIR) is a more potent α_2-receptor antagonist than the R(-) enantiomer (R-MIR), whilst the inverse is true regarding 5-HT_3-antagonism (246;249;250). Contrary to other chiral antidepressants, the differential receptor affinity profile of both MIR enantiomers seems to lead to an advantage of the racemate over a single enantiomer (199). Probably, the antidepressant effect of MIR resides mainly in the S-enantiomer, while the R-enantiomer may prevent some adverse effects such as nausea, due to stimulation of 5-HT3-receptors. A small study comparing cerebrospinal fluid (CSF) concentration with plasma levels of MIR and its enantiomers in MIR treated patients evinced much lower S-MIR than R-MIR CSF concentrations (251). Interestingly, Brockmöller et al (252) found enantioselective differences regarding adverse drug effects, with the effect of MIR on the heart rate and blood pressure correlating more strongly with the R-enantiomer than with the S-enantiomer.

Table 7: Pharmacological profile of mirtazapine with some enantioselective differences. In case of the α1- antagonistic property it is speculated that the R-enantiomer might be more potent since it is more associated with orthostatic hypotension and tachycardia.

receptor	mirtazapine
5HT2	Potent antagonist (S-enantiomer >> R)
5HT3	Potent antagonist (S-enantiomer << R)
H1	Potent antagonist
M	Moderate antagonist
α1	Moderate antagonist (S-enantiomer << R?)
Presynaptic α2	Antagonist (S-enantiomer >> R)

MIR kinetics is linear within the dose range 15 to 80 mg, but shows gender and age effects (253-257). Plasma levels in males are, independent of age, reportedly lower than those of females, and the plasma half-life is significantly shorter in adults than in the elderly (20 and 40 hours, respectively) (253). Renal and hepatic insufficiency can also result in substantially reduced clearance (253). Several isoforms of cytochrome P-450 (CYP) contribute in vitro to the enantioselective biotransformation of MIR: CYP1A2, CYP2D6 and CYP3A (258;259). For CYP2C19 no significant contribution has been found in vitro, a possible role of CYP2B6 has not been studied. The main metabolites are 8-hydroxymirtazapine (8-OH-MIR), N-desmethylmirtazapine (DMIR) and mirtazapine-N-oxide (MIR-N-oxide). N-demethylation and N-oxidation are catalysed by CYP3A (253;260). The 8-hydroxylation

process (followed by glucuronidation) is under the control of CYP2D6 and, to some extent, CYP1A2 (more important at higher MIR concentrations) and it is essentially associated with the S-enantiomer. For the R-enantiomer, (reversible) N-ammonium glucuronidation is the main metabolic step (259;261). A study in healthy volunteers did not suggest differences in the pharmacokinetics of MIR between extensive (EM) and poor metabolisers (PM) (CPY2D6) (260). However, a reanalysis of the samples using a stereoselective method showed that the elimination half-life of the R-enantiomer was longer than that of the S-enantiomer in EM (22.5 and 13.2 hours, respectively), and that the half-life of the S-enantiomer was increased in PM (18.8 hours) (253). A population pharmacokinetic analysis of MIR (262) found a distinct difference in clearance between CYP2D6 EM and intermediate metabolisers (IM), the clearance being reduced by 26% in IM. No other factor had a significant influence on MIR clearance. The similarity between CYP2D6 PM and EM might be related to a relatively lower importance of CYP2D6 in favour of CYP1A2 with increasing MIR exposure (262). One may then postulate that with low CYP2D6 activity, other pathways become more important.

Wide inter-individual variability in MIR plasma concentration in relation to dose has been found (255) and no drug plasma concentration-clinical effectiveness relationship has been demonstrated. The recommended therapeutic doses of 15-45 mg/d result in plasma levels ranging from 5-100 ng/ml (253); the recommended target range is 40-80 ng/ml (41).

The aim of this study was to evaluate the relationship between steady-state plasma concentrations of the enantiomers of MIR and its metabolites and the clinical effectiveness of MIR in moderately to severely depressed and CYP genotyped patients, including in elderly patients, taking their pharmacogenetic status into account.

Materials and Methods

Patients

This multicenter study recruited in- and out-patients (aged ≥18 years) with a primary diagnosis of major depressive episode (DSM-IV), unipolar or bipolar II (296.2, 296.3 or 296.89 according to the DSM-IV checklist), in one French (Besançon) and in 6 Swiss (Adult psychiatric and psychogeriatric university hospitals, Prilly-Lausanne; Königsfelden; Brig; Herisau; psychogeriatric university hospital Chêne-Bourg (Geneva)) psychiatric hospitals. Patients were scheduled according to age: 18-39 y (n = 20), 40-64 y (n = 30) > 64 y (n = 30). They were required to have a 17-item Hamilton Depression Rating Scale (HAMD) (263) total score of ≥18 points at baseline and a Mini-Mental State Examination MMSE (264) score of ≥24 at screening (day -3 to -1). Exclusion criteria included:

unacceptable severe cognitive impairment (defined as <24 on the MMSE); duration of current depressive episode >12 months; known or suspected active suicidal tendencies; a history of or current schizophrenia or organic mental disorders; current primary anxiety disorders (according to DSM IV), epilepsy, a history of seizure disorders or prior treatment with anticonvulsant medication for epilepsy or seizures; any clinically meaningful non-stable renal, hepatic, cardiovascular, respiratory or cerebrovascular disease or other serious progressive physical diseases; participation in other trials in the last 30 days; and pregnancy or lactation. Patients were also excluded if they had received MAO inhibitors of any type within 2 weeks of the start of treatment, fluoxetine within 5 weeks, and electroconvulsive therapy within 3 months or other psychotropic drugs within 2-3 days.

After procedures and possible side effects had been explained, all patients gave written informed consent prior to entering the study. The study was conducted according to Good Clinical Practices and the Declaration of Helsinki. In particular, the protocol was accepted by the corresponding local ethical committees. All investigators (one per centre) met twice for an inter rater's training.

Treatment

After a 3-day wash-out period, patients received oral MIR for 8 weeks. MIR was dispensed as 30 mg tablets to be taken as a single night-time dose of 30 mg/day on days 1-14 and 30-45 mg/day on days 15-56. The dose could not be modified between visits, but could be adapted on days 15, 28 and 42. Deviations from this dosing schedule, such as dose reductions below 30mg or at other time points, were only allowed in case of emergence of intolerable adverse events. Any unessential concomitant medication and the use of alcohol were discouraged. Concomitant medication for physical illnesses other than those specified by the exclusion criteria was permitted. In cases where sleeping problems persisted or were aggravated during the course of treatment, zopiclone (maximum 7.5 mg/day), zolpidem (maximum 10 mg/day) or chloral hydrate (maximum 2,000 mg/day) were allowed for night-time sedation. The following co-medications were not permitted: any other psychotropic drug, including short- and long-acting benzodiazepines (stable benzodiazepine users were allowed to remain on the same dose during the study - a maximum 30% change in dose was allowed); sedative drugs (including sedative antihistaminergics and antiemetics); antiepileptic drugs (including carbamazepine and valproate); and thyroid hormones.

For the evaluation of an interaction between MIR and co-medications, the interaction program used was: http://www.mediq.ch/ (retrieved March 2010).

Clinical assessments

Assessments were performed at screening (day -3 to -1), baseline (day 0) and days 7, 14, 21, 28, 42 and 56 of treatment or at endpoint. Clinical assessments comprised the 17-item HAMD (Hamilton

Depression Scale) (263) and the clinical global impression (CGI), vital signs, spontaneous adverse events, the UKU (Udvalg for Klinske Undersogelser) side effect rating scale (265), and smoking behaviour, alcohol and caffeine consumption. An ECG was recorded at screening and on day 56.

Biological assessments

Steady state trough plasma concentrations of the enantiomers of MIR, DMIR and 8-OH-MIR, were measured on days 14, 28, 42 and 56 using a recent stereoselective LC-MS method (200), after a 3-step extraction of the compounds. The limit of quantification (LOQ) for all enantiomers was 0.5ng/ml, and the intra- and inter-day coefficients of variation (CVs) were within 3.3% to 11.7% (concentration ranges 5-50 ng/ml). All plasma levels are expressed in ng/ml; for conversion in nmol/l: 1 ng/ml is equivalent to 3.774 nmol/l. Total (free and glucuroconjugated) concentrations of MIR and metabolites were also determined after submitting the plasma samples to enzymatic hydrolysis (hydrolysed samples). If not otherwise specified, drug concentrations mentioned in the text are those of non hydrolysed samples. As on day 14, all patients were medicated with the same mirtazapine dose (30mg/day), absolute plasma concentrations are given. For drug plasma concentrations measured on days 28, 42 or 56, dose corrected concentrations (ng/ml/mg dose) are presented or used for statistical comparisons.

At the end of the wash-out period, patients were phenotyped with dextromethorphan (CYP2D6) and mephenytoin (CYP2C19) (145). Dextromethorphan and its metabolite dextrorphan, and S- and R-mephenytoin were assayed in urine as previously described (145). Patients were also genotyped for CYP2D6 (alleles *1, *3, *4, *5, *6,*16 2XN (amplified)), CYP2C19 (alleles *1, *2, *3), CYP2B6 (alleles *1, *4, *5, *6, *7, *9) and CYP1A2 (allele *1F) as previously described (138). The patients were classified according to their CYP2D6 and CYP2C19 genotypes (ref. (138) and cf. Table 1): ultrarapid (UM), intermediate (IM), extensive (EM) and poor (PM) metabolisers.

Standard clinical chemistry (sodium, potassium, calcium, chlorine, inorganic phosphate, fasting glucose, total cholesterol, high-density lipoprotein (HDL)- cholesterol, triglycerides, albumin, total protein, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, γ-glutamyl transpeptidase, urea, creatinine, lactate dehydrogenase, total bilirubin, triiodothyronine (T3), thyroxine (T4), thyroid stimulating hormone (TSH)) and haematology (haemoglobin, haematocrit, mean corpuscular volume, erythrocytes, leucocytes, platelets) parameters were measured at baseline and at the end of the study.

Statistical analysis

The dependence of genotypes, sex, age and plasma levels of MIR enantiomers and metabolites were analyzed by the Kruskal Wallis test for >2 groups and the Mann Whitney test for two groups. All

values were reported as mean ± SD or median (minimum- maximum). A p-value < 0.05 was considered to indicate statistical significance. Confidence interval was 95%. Correlations were assessed by Spearman`s test. The same analysis was performed for the dependence of genotypes, HAMD total score, and change in HAMD score during the study, and plasma levels of MIR enantiomers and metabolites. In this exploratory study no corrections for multiple comparisons were made. Efficacy was assessed using the CGI and HAMD scales and the results were analyzed descriptively and graphically over time. The two-sample Wilcoxon test for paired data was applied to compare the CGI and HAMD scores at the beginning and end of the study. A responder was defined as a patient whose HAMD score fell by >50% compared with baseline at some time during the study. The rate of responders was displayed graphically over time both for the study completers and using the last observation carried forward (LOCF) method. Side effects measured with the UKU side effect scale were analyzed descriptively. Changes in weight and plasma levels of total cholesterol, HDL-cholesterol, triglycerides, glucose, T3, T4 and TSH over time were analyzed graphically and descriptively. The Wilcoxon test for unpaired data was applied to compare laboratory parameters in responders and non-responders. For detection of optimal cut-off values regarding responders/ non-responders receiver operating characteristic (ROC) curves were applied and the area under the curve (AUC) was calculated. For ROC- derived optimal cut-off values sensitivity, specificity, positive (PPV) and negative (NPV) predictive value were calculated. Optimal cut-off values are values corresponding with the highest accuracy. SPSS 16.0 (SPSS Inc. Chicago, IL) was used to perform the statistical analysis.

Results

A total of 45 patients (32 females (f)) were included in the study. The mean age was 51 years (range 19-79) and the age distribution was: 18-39 years n=13 (8 f), 40-64 years n=19 (13 f), and >64 years n=13 (11f). All patients were diagnosed with unipolar depression (13 with DSM296.2, 32 with DSM296.3). Twenty eight patients were non-smokers (22f), and 17 smokers (10f). One patient had a HAMD score of 17 at baseline (20 at screening), but was nevertheless included in the analyses because this protocol violation was considered non significant. Thirty one patients (69%) completed the study. The rate of study completion increased with age: 18-39 y: 54% (n=7); 40-64 y: 68% (n=13); > 64 y: 85% (n=11). Reasons for drop-out were loss to follow up (n=5), withdrawal of consent (n=5), protocol violation (n=1), inefficacy (n=1) and combined inefficacy/intolerability (n=2). A 37 year-old male patient was unable to tolerate the higher dose of 45 mg/d because of restless legs, sweating and agitation; he was switched back to the 30 mg dose, which proved ineffective, and then withdrawn from the study. Then 0.5 mg/d alprazolam and 5 mg/d olanzapine were added to his treatment with MIR.

Seven days later, during a family fight, he committed suicide by shooting himself. The investigators attribute causality for this suicide to the underlying disease that did not respond to treatment.

Co-medications

Anxiolytics/hypnotics were the most frequent co-medications: 58 times a drug of this class has been mentioned as co-medication, sometimes for the whole study period, sometimes for some days only; most frequently zolpidem, zopiclone and chloralhydrate for nighttimes' sedation, and lorazepam as anxiolytic stable co-medication. None of these drugs interferes pharmacokinetically with MIR. Cardiovascular drugs were co-prescribed 25 times. Twelve times an analgesic has been given, 3 times rofecoxib. In 2 patients rofecoxib was present on day 14. These patients showed higher plasma levels of S-MIR (median 17.4 ng/ml (15.9-18.9) versus 7.1 ng/ml (0.6-18.9), $p = 0.029$) and a higher ratio SMIR/SDMIR than the rest (median 9.9 (9.6-10.2) versus 2.6 (0.6-6.8), $p = 0.018$). Other medications included mainly vitamins, minerals, contraceptives, and drugs acting on the gastrointestinal tract. None of them were identified interacting with MIR metabolism.

Efficacy

All patients started their MIR treatment with 30mg/d for a minimum of 14 days. Thereafter the dose was either 30mg or 45mg daily; mean dose on day 56 was 38mg/d. The treatment resulted in a significant ($p < 0.0001$, Wilcoxon test) improvement in mean ± SD HAMD total score from 24.8 ± 4.9 at baseline to 9.8 ± 7.9 at the end of this open, not placebo controlled study (LOCF analysis). The response rate as assessed by the HAMD scale increased from 22.7% (confidence interval: 0.355, 0.099) at week 1 to 80.7% (confidence interval: 0.951, 0.662) at week 8, when 23 out of 31 patients were considered as responders. This clinical improvement was reflected by the CGI severity scale that showed a clear shift from mainly moderately to extremely ill patients at baseline to mainly not ill or borderline ill patients by the end of the study. Some patients showed a rapid improvement from week 1 onwards.

Drop-outs

14 patients dropped out during the study. Reasons and moments of drop out are found in table 8, further characteristics in table 9.

Table 8: Reason and moment of study drop out

Number of patients per reason of drop out	**Last visit**	**Remark**
1 protocol violation	Base line for clinical values, day 14	
3 inefficacy/intolerability	1 day 14, 2 day 28	
5 withdrawal of informed consent	2 day 7, 2 day 14, 1 day 21	2 (day 7) without laboratory
5 loss of follow up	1 day 7, 1 day 14, 2 day 28, 1 day 42	
14 Total (7 responder, 7 non responder)		

Table 9: Characteristics of study completers and dropouts.

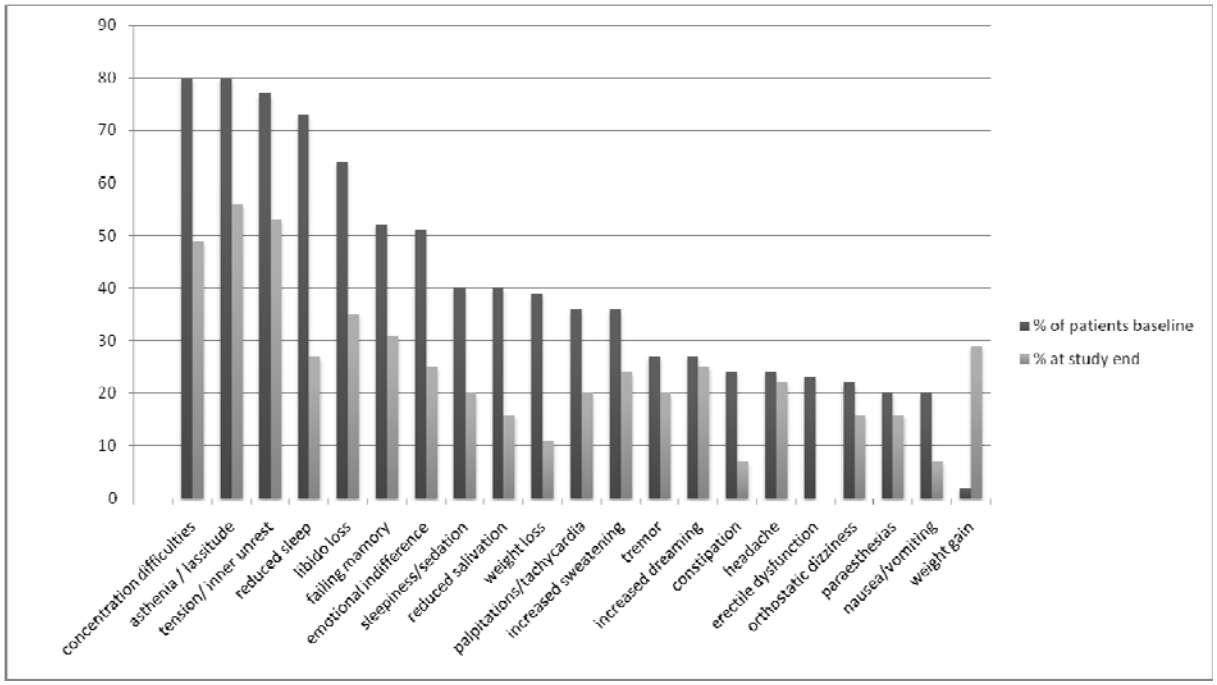
	Completers (n= 31)	**Dropouts (n=14)**
Mean age (years)	52.8	45.7
Sex	23f, 8m: f:m=2.9	9f, 5m: f:m=1.8
CYP1A2	4 *1/*1 =13% 7*1F/*1F 23%	3 *1/*1 =21% 6 *1F/*1F = 42%
CYP2B6	6 *6/*6 = 19%	2 *6/*6 = 14%
CYP2D6	1 PM = 3% 2UM = 6%	2 PM = 14% 1 UM = 7%

Tolerability and safety

No serious adverse drug reactions were reported during the study. The doctor's and patient's global assessments of side effects according to the UKU side effect rating scale were strikingly similar at all assessment points. Baseline and reported treatment emergent complaints were partly identical but at baseline more patients reported complaints than during the study period (fig. 11); > 50% suffered from asthenia/lassitude, concentration difficulties, tension/inner unrest, reduced sleep, sexual disturbances, failing memory, emotional indifference, all symptoms of a depressive illness. During the study the following adverse events, rated as possible or probable, were mentioned in >10% of the patients (by their decreasing frequency (LOCF)): asthenia/lassitude, weight gain, concentration difficulties, increased dream activity, headache, failing memory, increased sweating and decreased salivation. Side effects were mostly mild to moderate. Sedation and increased duration of sleep were more often reported at the start of treatment. Restless legs were spontaneously reported by 11% of the patients outside the UKU scale. When comparing side effects with baseline complaints weight gain was clearly associated to the treatment with MIR.

There was a weight gain during the study of 2.7 ± 2.9 kg ($p<0.001$; ANOVA Chi^2 test) (range -1.1 kg to 11.0 kg), with weight gains > 4 kg in 15.6%, and > 2 kg in 29% of the patients. The patient with a weight gain of 11 kg experienced deterioration of pre-existing dyslipidemia, dry mouth and restless legs, which were not associated with unusual plasma levels of MIR. There were four patients (9%) who had a weight gain of >10% during the study (a weight gain of >10%, according the AMSP criteria (16;266) (Arzneimittelsicherheit in der Psychiatrie (Drug Safety in Psychiatry)), is considered to be medically significant). There were no clinically significant overall changes in any laboratory parameters during the study.

Figure 11: Baseline and reported treatment emergent complaints as rated by the UKU scale



Cytochrome P-450 genotypes (Table 10) and phenotypes

None of the 45 genotyped patients presented a genetic deficiency of CYP2C19 (PM), but 9 and 36 subjects were classified as CYP2C19 IM (*CYP2C19 *1/*2*) or EM, respectively. The mephenytoin test demonstrated that all patients were EM or IM (as the mephenytoin test does not allow discriminating EM from IM). *CYP2D6* genotyping showed that 3 patients (6.7%) were PM, 3 patients were ultrarapid metabolisers (UM), 14 patients (31%) were IM, and 25 subjects were classified as EM. The phenotype predicted by the genotype was not congruent in all cases with the observed phenotype characterized by the dextromethorphan test. Four patients (2 IM and 2 EM by genotype) were identified as PM by phenotyping with dextromethorphan. Thirteen patients (29%) were homozygote for the *CYP1A2* allele *1F. In this study group, 14 subjects (31%) had the *1/*1 wild type *CYP2B6* genotype and 8 (18%) the *6/*6 genotype (Table 10).

Table 10. Cytochrome P-450 genotypes and the predicted phenotypes of the patients treated with mirtazapine (MIR)

Genotype		n	Frequency (%)	Predicted phenotype
CYP1A2	*CYP1A2*1F*			
	**1/*1*	7	15.6%	
	**1/*1F*	25	55.6%	
	**1F/*1F*	13	28.9%	
CYP2B6	*alleles *4/*5/*6/*7/*9*			
	**1/*1*	14	31.1%	
	**1/*4*	1	2.2%	
	**1/*5*	4	8.9%	
	**1/*6*	12	26.7%	
	**1/*7*	4	8.9%	
	**4/*6*	1	2.2%	
	**5/*5*	1	2.2%	
	**6/*6*	8	17.8%	
CYP2C19	*alleles *2/*3*			
	**1/*1*	36	80.0%	EM
	**1/*2*	9	20.0%	IM
CYP2D6	*alleles *3/*4/*5/*6/*16/*XN*			
	**1/*1*	24	53.3%	EM
	**1/*4*	10	22.2%	IM
	**1/*5*	4	8.9%	IM
	**1/*xN*	3	6.7%	UM
	**3/*4*	1	2.2%	PM
	**4/*6*	1	2.2%	PM
	**4/*xN*	1	2.2%	EM
	**5/*16*	1	2.2%	PM

Classification of the patients according to their genotypes and predicted phenotypes (35):
UM: ultrarapid metabolisers; IM: intermediate metabolisers; EM: extensive metabolisers;
PM: poor metabolisers

Pharmacokinetics

Only the pharmacokinetic data of day 14 will be presented here extensively, as all 45 patients were treated with the same daily dose of MIR (30 mg/day): Median (range) MIR plasma concentration reached 30.4 ng/ml (13.2-53.4) and in hydrolysed samples, this value increased drastically to 70.1 ng/ml (21.2-117.7). Complete data for plasma levels and geno- and phenotypes were available for 40 patients.

There were important stereoselective differences in the pharmacokinetics of MIR (Table 11). R-MIR and R-DMIR concentrations were about 2.8 and 6.5 times higher than their corresponding S-enantiomers, but this stereoselectivity almost disappeared when the enantiomers of OH-MIR were compared (when not otherwise specified, non-hydrolysed samples are meant). The situation was similar when hydrolysed samples were compared, except for the metabolite OH-MIR: the mean concentration of S-OH-MIR was almost 5 times higher than that of R-OH-MIR. While hydrolysis of the samples increased drastically the concentrations of the enantiomers of MIR and OH-MIR, this treatment had apparently only a small but nevertheless significant effect on R-DMIR ($p = 0.026$) but not S-DMIR (ns) plasma concentrations.

Table 11: Median (ranges) plasma concentrations (ng/ml) of the enantiomers of mirtazapine (MIR), desmethylmirtazapine (DMIR) and 8-hydroxymirtazapine (OH-MIR) on day 14 in CYP2D6 genotyped patients

	UM n = 3	EM n = 22	IM n = 13	EM+IM n = 35	PM n = 2	p (PM vs all other patients
Without hydrolysis						
R-MIR	20.8 (12.9-25.9)	22.1 (11.5-36.4)	22.0 (14.4-37.2)	22.0 (11.5-37.2)	18.7 (12.0-25.4)	0.576
S-MIR	10.3 (2.4-14.1)	6.6 (0.6-12.2)	9.4 (1.5-18.9)	6.9 (0.6-18.9)	13.3 (12.7-14.0)	0.082
R-MIR+S-MIR	31.1 (15.3-39.9)	27.8 (13.2-47.3)	34.4 (15.9-53.4)	28.9 (12.1-56.1)	32.0 (24.7-39.4)	0.756
R-DMIR	16.5 (15.9-41.7)	16.3 (7.3-28.2)	21.4 (7.4-30.2)	16.9 (7.3-30.2)	23.1 (18.9-27.3)	0.215
S-DMIR	1.5 (1.0-5.3)	2.2 (1.0-3.8)	3.3 (1.0-7.9)	2.6 (1.0-7.9)	4.2 (3.6-4.8)	0.133
R-OH-MIR	0.9	1.1 (0.5-5.8)	1.1	1.1 (0.5-5.8)	-	na
S-OH-MIR	0.8 (0.7-0.9)	0.9 (0.6-3.5)	1.2 (0.9-1.8)	1.1 (0.6-3.5)	1	0.957
With hydrolysis						
R-MIR	47.0 (30.4-62.0)	49.3 (19.1-85.4)	56.2 (22.7-87.3)	54.7 (19.1-87.3)	67.2 (46.6-87.9)	0.385
S-MIR	16.3 (3.9-26.9)	9.6 (2.1-20.6)	19.3 (3.2-30.4)	13.0 (2.1-30.4)	30.1 (29.6-30.5)	**0.030**
R-MIR+S-MIR	63.3 (34.3-88.9)	62.6 (21.2-98.4)	83.2 (25.9-117.7)	67.7 (21.2-117.7)	97.3 (77.0-117.5)	0.143
R-DMIR	13.0 (11.5-61.3)	11.6 (3.2-70.0)	13.6 (9.6-74.8)	12.3 (3.2-74.8)	21.8 (19.7-23.9)	0.172
S-DMIR	10.5	2.3 (1.4-2.6)	4.7 (2.4-7.5)	2.5 (1.4-7.5)	6.6 (3.8-9.5)	0.161
R-OH-MIR	7.8 (7.0-15.3)	6.8 (2.6-48.0)	3.5 (1.9-51.1)	6.2 (1.9-51.1)	8.6 (7.4-9.9)	0.484
S-OH-MIR	76.7 (74.6-100.1)	44.2 (15.6-90.6)	46.6 (15.7-110.8)	44.7 (15.6-110.8)	57.2 (55.1-59.2)	0.495

(*): UM: ultrarapid metabolisers; EM: homozygote extensive metabolisers; IM: heterozygote extensive metabolisers; PM: poor metabolisers

Plasma levels of MIR and metabolites were higher in non-smokers (n = 28) than in smokers (n = 17). Most comparisons reached statistical significance (median plasma levels (min-max), not dose corrected): S-MIR: 9.4 ng/ml (1.8-16.2) vs. 4.3 ng/ml (0.6-18.9) ($p = 0.014$); R-MIR: 24.1 ng/ml (11.5-37.2) vs. 18.6 ng/ml (12.2-27.8) ($p = 0.007$); S-DMIR 3.5 ng/ml (1.1-7.9) vs. 2.0 ng/ml (1.0-4.9) ($p = 0.006$); but R-DMIR 19.0 ng/ml (7.3-41.7) vs. 16.5 ng/ml (7.4-25.7) ($p = 0.053$; ns). The

ratio S-/R-MIR was higher in non smokers with 0.41(0.15-1.06) vs. 0.25(0.04-1.01) in smokers (p=0.025 Mann-Whitney). Such significant differences were not observed in hydrolysed samples (not shown).

Significant positive correlations were observed between age and plasma concentrations of MIR enantiomers and metabolites, in the samples submitted to hydrolysis: (S-MIR: r = 0.377 (p = 0.018); R-MIR: r = 0.576 (p = 0.0001); S-DMIR: r = 0.618 (p = 0.001); R-DMIR: r = 0.503 (p = 0.001); R-OH-MIR: r = 0.331 (p = 0.04)). Similar significant correlations were obtained at weeks 4, 6 and 8 (not shown). Inconsistent results were obtained by the statistical analysis of non-hydrolysed samples as at week 2, significant correlations were only observed for R-MIR: r = 0.398 (p = 0.01) and R-DMIR: r = 0.328 (p = 0.04), and only for R-MIR, significant correlations were also observed on weeks 4 and 8. Age and smoking status were not significantly correlated (r = -0.205, p = 0.177), and therefore the effect of smoking was not confounded by age.

The study of the relationship between gender and MIR kinetics (table 12) showed that female had significantly higher median R-MIR, R-DMIR and S-MIR plasma levels than male patients, both in samples without and with hydrolysis.

Table 12: Significant differences in plasma concentrations of MIR enantiomers and metabolites between female and male patients.

	non- hydrolysed			*hydrolysed*		
gender	R- MIR	R- DMIR	S- MIR	R- MIR	R- DMIR	S- MIR
female	23.46±6.87	20.23±7.25	9.47±4.91	57.36±16.75	23.29±21.17	16.47±9.00
male	19.31±3.39	15.00±4.88	5.79±3.89	43.21±18.81	13.97±10.58	10.34±6.42
p	0.029	0.015	0.029	0.022	0.024	0.035

Pharmacokinetics – pharmacogenetics relationships

Relationships between the pharmacogenetic status of the patients regarding *CYP2C19* polymorphisms and plasma concentrations of MIR and its metabolites as measured on day 14 (but also at weeks 4, 6, and 8) were calculated. There was apparently no evidence for a direct effect of the *CYP2C19* pharmacogenetic status of the patients on MIR kinetics (not shown).

A statistically significant influence of the *CYP2D6* genotype (PM, IM, EM, UM) on plasma levels of MIR enantiomers or metabolites was only observed for S-DMIR plasma concentrations at almost all data points (p= 0.09, 0.014, 0.02, 0.006 respectively) after 2, 4, 6, and 8 weeks (data not shown).

Ratios between the concentrations of the parent compound and the metabolite, as well as between the enantiomers, which could be formed by a particular enzyme, were calculated in order to examine

pharmacogenetic relationships (table 13): In the whole patient population, the ratios S-MIR/S-OH-MIR (p = 0.014) and S-MIR/R-MIR (p = 0.043) in the hydrolyzed samples showed a statistically significant *CYP2D6* dependence, S-DMIR/R-DMIR showed a trend (Kruskal Wallis test, data not shown). Analyzing smokers and non-smokers separately, these effect could only be confirmed in non-smokers (S-MIR/S-OH-MIR (p = 0.023); S-MIR/R-MIR (p = 0.015)) (Table 14a and b).

Table 13: Median (ranges) ratios of the enantiomers of MIR, DMIR and OH-MIR on day 14 in plasma of CYP2D6 genotyped patients treated with mirtazapine 30mg/d.

	UM n = 3	EM n = 22	IM n = 13	EM+IM n = 35	PM n = 2	p (PM vs all other patients
Without hydrolysis						
S-MIR/R-MIR	0.50 (0.19-0.55)	0.27 (0.04-0.45)	0.41 (0.10-1.01)	0.30 (0.04-1.01)	0.81 (0.55-1.06)	**0.030**
S-MIR/S-DMIR	2.67 (2.35-6.75)	2.48 (0.64-6.40)	2.19 (1.19-9.56)	2.43 (0.64-9.56)	3.22 (2.94-3.50)	0.647
R-MIR/R-DMIR	0.81 (0.62-1.26)	1.38 (0.61-2.50)	1.12 (0.73-1.96)	1.32 (0.61-2.50)	0.89 (0.44-1.34)	0.321
S-MIR/S-OH-MIR	11.23 (3.49-17.61)	7.38 (1.36-15.41)	7.40 (2.56-22.01)	7.39 (1.36-22.01)	13.21	0.176
R-MIR/R-OH-MIR	29.71	22.70 (6.02-40.55)	24.35	24.35 (6.02-40.55)	-	na
With hydrolysis						
S-MIR/R-MIR	0.35 (0.13-0.43)	0.21 (0.11-0.32)	0.31 (0.10-0.56)	0.24 (0.10-0.56)	0.50 (0.34-0.65)	0.056
S-MIR/S-DMIR	2.57	5.27 (2.17-11.26)	4.16 (1.77-8.94)	4.95 (1.77-11.26)	5.56 (3.13-7.98)	1.000
R-MIR/R-DMIR	2.34 (1.01-4.09)	4.13 (0.71-11.67)	3.88 (1.05-5.63)	4.01 (0.71-11.67)	3.21 (1.94-4.47)	0.710
S-MIR/S-OH-MIR	0.16 (0.05-0.36)	0.19 (0.08-0.45)	0.35 (0.16-0.67)	0.26 (0.08-0.67)	0.53 (0.50-0.55)	**0.036**
R-MIR/R-OH-MIR	4.06 (3.89-6.73)	7.61 (1.24-13.06)	12.76 (1.54-33.76)	8.51 (1.24-33.76)	7.62 (6.33-8.91)	0.799

(*): Abbreviations: cf Table 1

Table 14a: Median (ranges) concentrations and ratios of the enantiomers of MIR, DMIR and OH-MIR on day 14 in non-hydrolysed plasma samples of CYP2D6 genotyped patients treated with mirtazapine 30mg/d: smokers vs. non- smokers.

	UM	EM	IM	PM	p Kruskal-Wallis
Smokers	n = 1	n = 8	n = 7		
R-MIR	12.7	19.3 (12.2-23.9)	18.6 (14.4-26.0)	-	0.370
S-MIR	2.4	3.4 (0.6-7.2)	5.9 (1.5-18.9)	-	0.242
R-DMIR	15.9	15.4 (8.8-25.7)	16.9 (7.4-22.2)	-	0.599
S-DMIR	1.0	2.1 (1.0-3.1)	2.8 (1.0-4.9)	-	0.242
R-OH-MIR	-	1.2	-	-	na
S-OH-MIR	0.7	1.1 (0.6-1.3)	1.2 (0.0-1.4)	-	0.198
S-MIR/S-OH-MIR	3.5	5.9 (3.0-9.0)	4.5 (2.6-22.0)	-	0.612
R-MIR/R-OH-MIR	-	12.1	-	-	na
Non-smokers	n = 2	n = 14	n = 6	n= 2	
R-MIR	23.3 (20.8-25.9)	23.4 (11.5-36.4)	24.7 (21.4-37.2)	18.7 (12.0-25.4)	0.716
S-MIR	12.2 (10.3-14.1)	7.1 (1.8-12.2)	10.4 (6.9-16.2)	13.3 (12.7-14.0)	**0.031**
R-DMIR	29.1 (16.5-41.7)	16.9 (7.3-28.2)	23.0 (12.1-30.2)	23.1 (18.9-27.3)	0.258
S-DMIR	3.4 (1.5-5.3)	2.3 (1.1-3.8)	4.3 (3.1-7.9)	4.2 (3.6-4.8)	**0.025**
R-OH-MIR	0.9	1.0 (0.5-5.8)	1.1	-	0.670
S-OH-MIR	0.9 (0.8-0.9)	0.9 (0.6-3.5)	1.3 (0.9-1.8)	1.0	0.451
S-MIR/S-OH-MIR	14.4 (11.2-17.6)	7.7 (1.4-15.4)	9.2 (7.1-10.2)	13.2	0.093
R-MIR/R-OH-MIR	29.7	33.3 (6.0-40.1)	24.4	-	0.766

(*): Abbreviations: cf Table 1; na: not analysed

Table 14b: Median (ranges) concentrations and ratios of the enantiomers of MIR, DMIR and OH-MIR in hydrolysed plasma samples of CYP2D6 genotyped patients treated with mirtazapine 30mg/d: smokers vs. non-smokers

	UM	EM	IM	PM	p Kruskal-Wallis
Smokers	n = 1	n = 8	n = 7		
R-MIR	30.4	52.9 (22.5-85.4)	43.6 (22.7-56.2)	-	0.320
S-MIR	3.9	9.6 (5.5-16.7)	8.2 (3.2-30.0)	-	0.381
R-DMIR	13.0	12.7 (6.1-67.7)	10.4 (9.6-32.6)	-	0.906
S-DMIR	-	2.2 (1.5-2.6)	3.6 (2.4-5.9)	-	0.086
R-OH-MIR	7.8	12.3 (6.1-48.0)	3.5 (1.9-20.1)	-	**0.048**
S-OH-MIR	76.7	53.7 (16.2-90.6)	29.8 (15.7-72.4)	-	0.274
S-MIR/S-OH-MIR	0.1	0.2 (0.1-0.5)	0.3 (0.2-0.6)	-	0.088
R-MIR/R-OH-MIR	3.9	3.5 (1.8-11.0)	12.8 (2.2-19.5)	-	0.052
Non-smokers	n = 2	n = 14	n= 6	n= 2	
R-MIR	54.5 (47.0-62.1)	48.6 (19.1-75.9)	73.0 (59.6-87.3)	67.2 (46.6-87.9)	**0.040**
S-MIR	21.6 (16.3-26.9)	9.9 (2.1-20.6)	23.1 (13.5-30.4)	30.1 (29.6-30.5)	**0.003**
R-DMIR	36.4 (11.5-61.3)	11.0 (3.1-70.0)	16.5 (11.1-74.8)	21.8 (19.7-23.9)	0.108
S-DMIR	10.5	2.3 (1.4-2.6)	5.5 (3.4-7.5)	6.6 (3.8-9.5)	**0.008**
R-OH-MIR	11.1 (7.0-15.3)	5.8 (2.6-46.5)	5.4 (2.0-51.1)	8.6 (7.4-9.9)	0.582
S-OH-MIR	87.3 (74.6-100.1)	41.2 (15.6-81.7)	55.1 (39.6-110.8)	57.2 (55.1-59.2)	0.087
S-MIR/S-OH-MIR	0.3 (0.2-0.4)	0.2 (0.1-0.4)	0.4 (0.3-0.7)	0.5 (0.5-0.6)	**0.023**
R-MIR/R-OH-MIR	5.4 (4.1-6.7)	9.5 (1.2-13.1)	14.2 (1.6-33.8)	7.6 (6.3-8.9)	0.517

(*): Abbreviations: cf Table 1

For *CYP1A2*F* we found some significant influence on day 14 for D-MIR (p = 0.039), in smokers for the ratio S-DMIR/R-DMIR (p = 0.039), and in non-smokers for the ratio R-MIR/R-DMIR (p = 0.047). For the distribution of CYP1A2*1F genotypes cf. table 15.

*Table 15: CYP1A2*1F genotypes in smokers and non smokers*

	CYP1A2			
	***1/*1**	***1/*1F**	***1F/*1F**	**total**
smoker	1	11	5	17
non smoker	6	14	6	31

One patient treated throughout the study with 30 mg/day MIR had consistently high plasma levels of enantiomers of MIR and its metabolites. Her racemic MIR plasma levels rose steadily from 41ng/ml after 2 weeks to 91ng/ml after 8 weeks. Her dose-corrected plasma MIR levels rose steadily from <1.5 ng x day /ml x mg after 2 weeks of treatment to 3.0 ng x day /ml x mg after 8 weeks. In contrast, the remaining patients had dose-corrected levels of <1.5 ng x day /ml x mg at almost all time points for the entire duration of the study. She weighed 48 kg, was 157 cm tall and smoked around 20 cigarettes per day. She was an ex-alcohol abuser and had high levels of liver enzymes (γ-GT consistently >100 U/L). As well as receiving MIR, at a dose of 30 mg/day, she was also taking concomitant vitamin B, calcium, acamprosate and propranolol. The fact that propranolol is a moderate inhibitor of CYP2D6

(267) may have contributed to the higher than expected MIR plasma levels. Her genotype for *CYP2D6* was determined as *IM *1/*5* (*5 a deficient allele); however her observed phenotype as characterized by the dextromethorphan test was not a PM which is against the hypothesis above. Her other genotypes were: *CYP1A2 *1/*1F, CYP2B6 *6/*6, CYP2C19 *1/*1*.

Patients with the genotype *CYP2B6 *6/*6* (n=8) compared with those presenting another *CYP2B6* genotype (n = 37) had similar plasma concentrations of R-MIR: 24.0 ng/ml (16.2 -37.2) (median, range) vs. 22.0 ng/ml (11.5-36.4) and S-MIR: 7.2 ng/ml (2.6-18.9) vs. 7.2 ng/ml (0.6-18.9), but somewhat higher levels of R-DMIR: 21.9 ng/ml (8.8-25.3) vs. 16.7 ng/ml (7.3-41.7); S-DMIR: 3.2 ng/ml (1.3-4.4) vs. 2.6 ng/ml (1.0-7.9); R-OH-MIR: 5.8 ng/ml (5.8) vs. 1.0 ng/ml (0.5-1.2); they were significantly higher only for S-OH-MIR (1.7 ng/ml (1.2-3.5) vs. 0.9 ng/ml (0.6-1.6) (p = 0.01)). In patients displaying the *CYP2B6 *6/*6* genotype, the sum of the two enantiomers of MIR (and that of its metabolites) were also somewhat higher but without reaching statistical significance (results not shown).

Pharmacokinetics, pharmacogenetics, clinical outcome

On day 56, at the end of the 8-week study period, the mean dose of mirtazapine was 38.5 ± 7.6 and 37.5 ± 8.0 mg/day in responders (n = 23) and non responders (n = 8) respectively. The plasma (not dose corrected) concentrations (median (min-max)) of MIR and its metabolites were: R-MIR: 25.2 ng/ml (15.0-65.2) and 25.1 ng/ml (8.5-40.3), S-MIR: 9.7 ng/ml (0.8-49.1) and 5.8 ng/ml (0.5-14.2), R-MIR + S-MIR: 34.9 ng/ml (17.4- 108.2) and 31.6 ng/ml (9.0-54.5). There was no evidence for a significant plasma concentration – clinical effectiveness or tolerability relationship regarding any pharmacokinetic parameter. Although plasma concentrations of the enantiomers of MIR and MIR metabolites did not differ between responders and non-responders, a ROC-analysis of our data indicates that patients with a plasma concentration of S-MIR ≥ 5 ng/ml will be responders with a probability of 77% (sensitivity 91%, specificity 50%). Also in the Chi square test this difference is statistically significant (table 16).

Table 16: Study completers divided in responders and non responders and S-MIR plasma concentration. Chi square is 4.64(p= 0.031)

S-MIR	Responders	Non responders	All
> 4.96ng/ml	20	4	24
< 4.96ng/ml	3	4	7
Total	23	8	31

With the exception of *CYP2B6* there were no significant relationships between *CYP* genotypes, HAMD total scores or change in HAMD total scores. Patients with the *CYP2B6 *6/*6* genotype had a higher reduction in HAMD scores (p = 0.016, Mann-Whitney test) than the patients presenting another *CYP2B6* genotype (Table 17).

Table 17: Association between CYP2D6 genotype and HAMD scores at baseline/study end

	Base HAMD	End HAMD	% change
CYP2B6 *6/*6	27.5 (19-34) n= 8	3 (0-4) n= 6	89.3 (80-100) n= 6
CYP2B6 Rest	24 (18-35) n= 37	8 (0-28) n= 25	66.7 (0-100) n=25
P value	0.166	0.018	0.016

Discussion

Clinical outcome

Although this was not a blind, placebo controlled study design, the results confirm the clinical response of MIR in patients (19-79 years old, 13 being >64 years old) suffering from an episode of major depression, resulting in a significant improvement in HAMD and CGI severity score (240;268). In this 8-week study, there was a mean decrease of 15 points on the HAMD scale, in comparison to a mean 11.5 points decrease found in a meta-analysis of 5 randomized, double-blind trials with 5 – 60 mg/d MIR administered during 5 – 6 weeks (240). In another analysis of MIR studies in patients with moderate to severe depression, response rate varied from 51 to 80 % after a 4 – 7-week treatment (240) – the response rate was 81% in the present study. The tolerability and safety profile was also as expected, with sedative effects and body weight increase being the most common adverse events. One patient committed suicide some days after being withdrawn from the study because of lack of therapeutic effect. Although in some cases a causal relation to antidepressant treatment has been suggested, this was not the case with this patient, and furthermore a recent analysis of 15 pooled studies with mirtazapine showed no increased but a lowered risk (269).

Cytochrome P-450 genotypes/phenotypes

The patients were phenotyped and/or genotyped for some (CYP2D6, CYP1A2) but not for all (CYP3A) enzymes known to be implicated in the metabolism of MIR (258;260). In particular, it was found that the percentage of patients (29%) homozygote for the *CYP1A2* allele *1F was lower than expected from the incidence of around 50% seen in the general population (70), but that could be a chance finding due to the low number of our study population. Homozygote subjects for this allele might show higher inducibility of CYP1A2 (70). The allele frequency for *CYP2C19*2* was low in our population with no PM, neither by genotype nor by phenotype (64;140;270). This makes it difficult to identify a possible effect of this enzyme but anyway, available literature on this subject does not suggest a relevant role of CYP2C19 in the metabolism of MIR (258;259). Regarding CYP2D6, no co-medication was identified that could sufficiently explain the difference between the predicted and observed phenotypes. It is known that the UM phenotype cannot be identified by the dextromethorphan test, and genotyping reveals only about 30% of UM (271;272). For statistical comparisons with clinical and pharmacokinetic parameters, only genotyping data were used. On the other hand, for the first time, a possible contribution of CYP2B6 in the biotransformation of MIR has been examined in this study, where 15% of the 45 patients presented the genotype *CYP2B6 *6/*6* (*CYP2B6 G to T* polymorphism at position 516).

MIR pharmacokinetics

The concentrations of the enantiomers of MIR and its metabolites (Tables 11-14) can only to a limited extent be compared with data from the literature. All previous measurements of the enantiomers of MIR and its metabolites in steady-state conditions were performed by the same laboratory, using the same method ((200;251;257), as in this study. In all these investigations, R-MIR concentrations were generally higher than those of S-MIR, both in hydrolysed and non-hydrolysed samples (including in those from CYP2D6 PM), except in those from patients co-medicated with CYP2D6 inhibitors such as fluoxetine (200). However, it seems probable, that the antidepressant effect of MIR is mainly exerted by S-MIR, due to its preferential affinity for α2-receptors (243;246;250).

This study also confirms that R- and S-MIR, R- and S-OH-MIR are largely conjugated (200;251), but as these conjugated products were apparently never synthesised as pure compounds, no data are available on their pharmacological profile and therefore, on their possible clinical contribution.

Mainly after hydrolysis, significant positive correlations were observed between almost all drug or metabolite levels and age and as well non-conjugated as total drug or metabolite concentrations were significantly higher in females than in males. The data suggest that these age and gender effects may probably be more pronounced with regard to the pharmacokinetics of the R-enantiomer. In the already mentioned study which reported only non-conjugated levels of MIR and DMIR (257), the

concentrations of the enantiomers of MIR and DMIR were also found to be higher in elderly (> 65 y) than in younger patients, but on the other hand, these levels only tended to be higher in females than in males. Finally, in the therapeutic drug monitoring study (with the same patients as reported in (257)), where only achiral methods were used, both higher age and female gender were related with higher levels of dose corrected MIR and DMIR (256). In summary, after taking into account also some single dose studies (273), higher plasma concentrations of MIR and its metabolites are rather expected in elderly patients and in female subjects, but the mechanism is not clear (metabolism by cytochrome P-450 isozymes or conjugating enzymes, liver blood flow, renal elimination) (253).

Smokers had significantly lower plasma concentrations of S-MIR ($p = 0.014$), R-MIR ($p = 0.007$) and S-DMIR ($p = 0.006$) than non-smokers, their ratio S-/R-MIR was lower as well ($p=0.025$). These findings, to some extent, are in line with previous publications (257). Using an achiral method, dose corrected MIR and DMIR concentrations were found to be lower in smokers than in non-smokers in the 6-month therapeutic drug monitoring study (256). A reanalysis of the samples of this investigation (256) by a stereoselective method (200) revealed that S-MIR ($p = 0.026$) and R-DMIR ($p = 0.036$), as well as the ratio S-/R-MIR were lower in smokers than in non-smoking patients (257). This suggests that CYP1A2, which is induced by smoke (274), contributes to the biotransformation of MIR. However, as already discussed earlier (256;257), in vitro studies with recombinant enzymes suggest that CYP1A2 may dose-dependently be involved in N-demethylation, hydroxylation and N-oxidation of MIR (258). In another in vitro study, limited methodologically by the fact that only the decrease of substrate but not the formation of a metabolite was measured, CYP1A2 preferentially but only marginally (in comparison to CYP2D6) metabolised S-MIR (S-(+)-MIR), while it was found to be inactive towards R-(-)-MIR (259).

Only few of co-medicated drugs can be considered as potentially interfering with the metabolism of MIR: The 2 β -blocking drugs metoprolol (275) and propranol (267) have some inhibitory potential on CYP2D6, and rofecoxib inhibits CYP1A2 activity (276;277). In 2 patients co-medicated with this analgesic drug, which in the meanwhile is not any more available on the market, S-MIR concentrations were higher than in the other patients ($p = 0.026$). Actually, in a patient treated with MIR (200), co-medication with several psychotropic and somatic drugs including omeprazole (a CYP1A2 inducer and CYP2C19 inhibitor) and rofecoxib, a ratio S/R-MIR = 9.4 could be observed in plasma, which seems to be the highest ratio ever reported.

Pharmacokinetic-pharmacogenetic relationships

While these comparisons between the smoking status of the patients and drug plasma concentrations show the influence of environmental factors on the stereoselective biotransformation of MIR, genetic factors also play an important role, which may be partly masked in smokers. Indeed, considering the

non-hydrolysed samples only, there is some evidence of a control of the metabolism of S-MIR (p = 0.031) and S-DMIR (p = 0.025) by CYP2D6 (Table 14a) only in non-smokers. Probably due to the low number of CYP2D6 PM, the ratio S-MIR/S-OH-MIR only shows a tendency to vary with the pharmacogenetic status of the patients (p = 0.093). These relationships appear more clearly when total (conjugated and non-conjugated) concentrations of S-MIR (p = 0.003), S-DMIR (p = 0.008) and S-MIR/S-OH-MIR (p = 0.023) are considered (Table 14b). In addition, CYP2D6 also contributes to some extent to the metabolism of R-MIR (p = 0.04). Again, CYP2D6 effects were stronger in non-smokers. These observations confirm those obtained earlier in 95 patients, which showed a relationship between the CYP2D6 genotype and S-MIR concentrations and with the S/R-ratio (257).

The MIR plasma concentrations from the 3 UM (CYP2D6) did not significantly differ from the EM (CYP2D6). It might be assumed that UM undergo a risk of therapeutic failure due to low tissue concentrations as CYP2D6 accounts for about a third to half of their total MIR biotransformation (278). However, of the 3 UM in this study, two responded well and the third withdrew prematurely. Similarly, in a previous study conducted in healthy volunteers, the impact of this genotype on MIR pharmacokinetics was less than expected (278). Definite conclusions cannot be drawn due to the low number of subjects, but S-MIR in the only smoking UM was considerably lower than in the 2 UM non-smokers, both in hydrolysed and non-hydrolysed plasma samples (Tables 14 a and b). This supports again the hypothesis that CYP1A2 activity, mainly in smokers, masks that of CYP2D6.

*CYP1A2*1F* has been associated with increased CYP1A2 activity in smokers, possibly because of increased inducibility (69;70). The *CYP1A2*F* polymorphism believed to enhance the inducibility of CYP1A2 did show some significant influence on enantiomeric MIR and metabolites plasma concentrations. However, these findings are difficult to interpret, since also non-smokers seemed to show some *CYP1A2*F* genotype dependency; but also for clozapine, mainly metabolised by CYP1A2, findings were contradictory (71;182;279).

The finding that the plasma concentrations of S-OH-MIR are significantly higher (p = 0.01) in patients presenting the genotype *CYP2B6 *6/*6* (*CYP2B6 G to T* polymorphism at position 516) than in the other patients is highly interesting, however also difficult to interpret. It is to note that the concentrations of the other metabolites were also higher in the *CYP2B6 *6/*6* patients, but these differences did not reach statistical significance, possibly due to the low number of tested subjects. Nevertheless, this suggests the hypothesis that in *CYP2B6 *6/*6* patients, metabolism of S- and possibly also of R-MIR is enhanced, but the question remains open whether both hydroxylation and N-demethylation are concerned. Only a few other psychotropic drugs were as yet shown to be substrates of CYP2B6, including bupropion (93), sertraline (280;281) and methadone (with a stereoselectivity in favour of S-methadone) (138). Most often, the presence of the *CYP2B6 *6/*6* genotype is

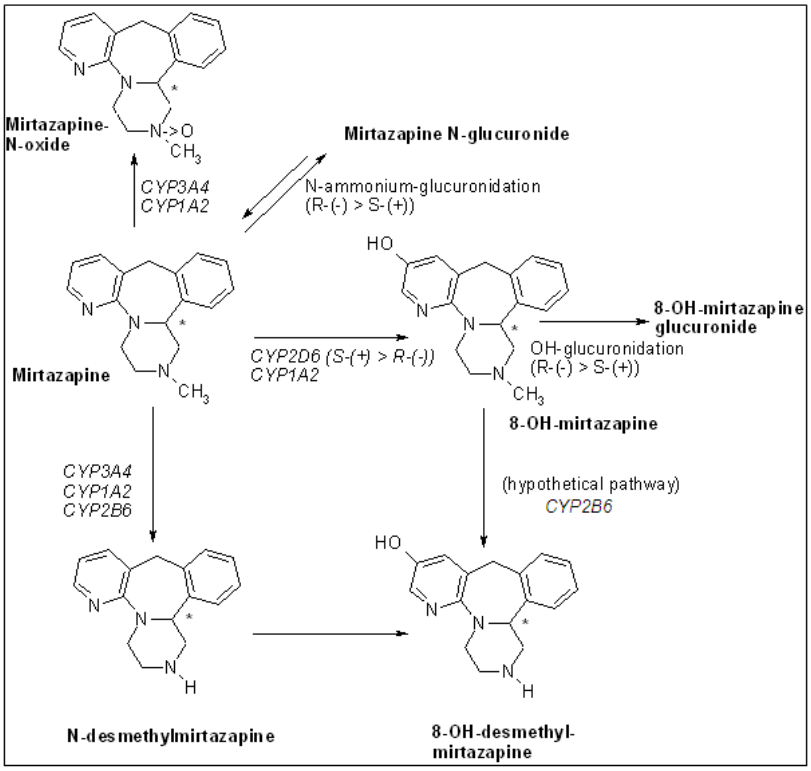
synonymous with decreased enzymatic activity as a consequence of a decreased enzyme protein expression, but with some substrates, increased metabolism has also been observed (282).

MIR pharmacokinetics and pharmacogenetics relationship with clinical outcome

Steady-state plasma concentrations of the enantiomers of MIR and its metabolites were dependent on age, gender, smoking, and they were related with the pharmacogenetic status of the patients regarding *CYP2D6* and probably also *CYP2B6* genotypes, but not regarding *CYP2C19* or *CYP1A2*F* genotypes. However, there was no significant relationship between plasma concentrations of MIR or its metabolites, or the pharmacogenetic status of the patients and clinical outcome, except that patients presenting the *CYP2B*6/*6* genotype had a better clinical response than the other patients (Table 17). The question arises if S-OH-MIR contributes to the therapeutic effect of MIR. No similar studies were previously published, but a clinical study with depressive patients treated with MIR for a varying period suggests that MIR plasma concentrations < 30 ng/ml represent a risk for decreased response(283). Unfortunately, no enantioselective methods were used to allow comparison with the present study, where concentrations of S-MIR > 5 ng/ml were synonymous with a higher response rate. Although recommended doses of MIR were given in this study, mean or median plasma concentrations hardly met the recommended target of 40-70ng/ml (41).

In summary, several forms of cytochrome P-450 are involved stereoselectively in the metabolism of MIR: CYP2D6, CYP3A, CYP1A2 and, as shown in this study, possibly also CYP2B6. Dose dependently, the contribution of the various enzymes varies, but also in relationship with the pharmacogenetic status of the patient (CYP2D6, CYP2B6), his phenotype with respect to CYP1A2 (e.g. induction by smoking) and probably also to CYP3A; gender and age affect also MIR plasma concentrations. Hedlund et al. (284) formulated the question: "2B or not 2B" with regard to its possible presence in the brain. This question is also justified considering the preliminary findings in this study about the metabolism of MIR by CYP2B6. In order to clarify this very complex situation, in vitro studies would be helpful as those carried out for sertraline (280) and clozapine (181), which allowed presenting the relative activity of each CYP isoform in function of the drug dose. In figure 12 a hypothesis is presented, how these different CYP450 enzymes could be involved in the metabolism of mirtazapine. Studies with larger groups of patients are needed in order to ascertain the role of CYP2B6 in the metabolism of MIR and its implication in the clinical response of the patients to this antidepressant. Indeed, the relatively small study size must be borne in mind and it is possible that a larger sample size may reveal more significant correlations. Moreover, neither *CYP3A* genotypes nor phenotypes have been examined and, in addition to rofecoxib and some β-blockers, other co-medications may also have influenced MIR pharmacokinetics.

Figure 12: Possible metabolic pathways of mirtazapine



5 Pharmacogenetic study of clozapine [1]

Summary

In order to examine the genetic factors influencing clozapine kinetics in vivo, 75 patients treated with clozapine were genotyped for CYPs and ABCB1 polymorphisms, and phenotyped for CYP1A2 and CYP3A activity. CYP1A2 activity and dose corrected trough steady-state plasma concentrations of clozapine correlated significantly ($r=0.61$; $p=1x10^{-6}$), with no influence of the CYP1A2*1F genotype ($p=0.38$). CYP2C19 poor metabolisers (*2/*2 genotype) had 2.3-fold higher ($p=0.036$) clozapine concentrations than the extensive metabolisers (non *2/*2). In patients co-medicated with fluvoxamine, a strong CYP1A2 inhibitor, clozapine and norclozapine concentrations correlated with CYP3A activity ($r=0.44$, $p=0.075$; $r=0.63$, $p=0.007$, respectively). Carriers of the ABCB1 3435TT genotype had 1.6 fold higher clozapine plasma concentrations than non-carriers ($p=0.046$). In conclusion, this study showed for the first time a significant role of CYP2C19 and the Pgp transporter in the in vivo pharmacokinetics of clozapine. CYP1A2 is the main CYP isoform involved in clozapine metabolism, with CYP2C19 contributing moderately, and CYP3A4 contributing only in patients with reduced CYP1A2 activity. In addition, ABCB1, but not CYP2B6, CYP2C9, CYP2D6, CYP3A5 nor CYP3A7 polymorphisms, influence clozapine pharmacokinetics.

Introduction

The first atypical antipsychotic drug clozapine is still considered superior in its efficacy to that of other antipsychotics (285), but because of its side effect profile (with amongst other the risk of severe haematological problems) it is mainly used in difficult to treat patients who failed to respond to other medication. However, about 30% of the patients do not respond adequately (286), one of the reasons could be too low plasma levels in spite of normal dosing. The well defined therapeutic window of clozapine of 350 – 600 ng/ml has been found in several studies (287;288); high plasma concentrations (>1000 ng/ml) are associated with a higher risk for serious adverse reactions such as generalised seizures, delirium, confusion (289).

The therapeutic effect of the drug clozapine is mainly attributed to the parent compound but the main active metabolite N-desmethyl clozapine (norclozapine) might have antipsychotic properties as well and beneficial effects on cognition (290;291) attributed to the M1 partial agonistic activity. In some studies a higher metabolic ratio of norclozapine/clozapine showed to be associated to a higher response rate (292;293). Norclozapine is, as the antipsychotic aripiprazole, a partial agonist for D2 and D3 receptors (294) which could explain the low incidence of EPS and maybe also positive effect on

[1] This study contributed also to the medical thesis of Branka Knezevic 2009

the negative symptoms of schizophrenia. Clozapine N-oxide seems not to have a significant clinically relevant activity (291;293;295;296). A comparison of the pharmacological profile of clozapine and norclozapine shows many similarities but also some striking differences (table 18).

Table 18: Pharmacological profiles of clozapine and norclozapine (291;293;297;298)

receptor	clozapine	norclozapine
D2	**inverse agonist/antagonist**	**middle potent partial agonist**
D3	**inverse agonist/antagonist**	**middle potent partial agonist**
D4	antagonist	antagonist
5HT1a	weak partial agonist	weak partial agonist
5HT2a	inverse agonist, high affinity	inverse agonist, high affinity
5HT2c	**inverse agonist**	**inverse agonist, high affinity, higher potency**
5HT6	middle potency inverse agonist,	middle potency inverse agonist, high affinity
5HT7	middle potency inverse agonist, high	middle potency inverse agonist, high affinity
H1	high potency inverse agonist	inverse agonist, but << than clozapine
H3	antagonist, middle affinity	antagonist, low affinity
M1	**antagonist**	**potent partial agonist**
M2	agonist	partial agonist
M3	-	partial agonist
M4	agonist	partial agonist
M5	-	partial agonist
α1A	antagonist, high affinity	antagonist, but lower affinity than clozapine

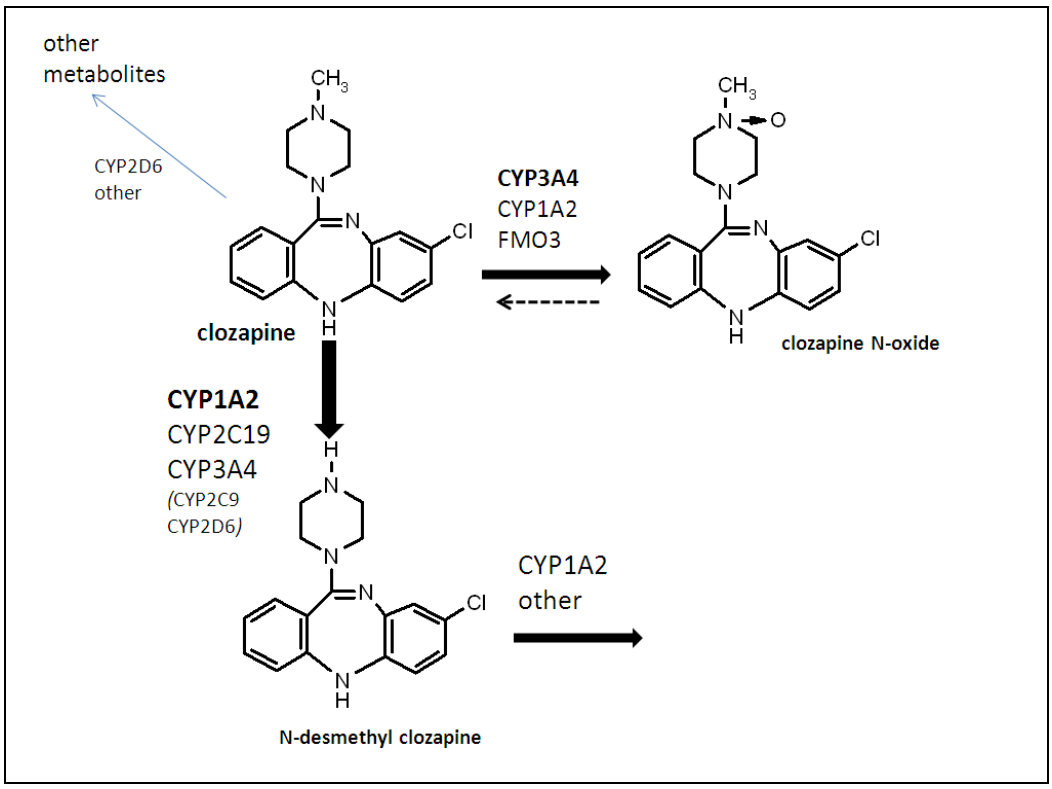
Considering side effects, M1 agonistic activity of norclozapine explains the observed sialorrhea, the M2 antagonism of the parent compound clozapine the relative protection against extrapyramidal symptoms. The H1 antagonistic activity of clozapine and norclozapine could explain the weight gain observed with clozapine, clozapine being more potent. This seems contradictory to the clinical observations of less weight gain with lower norclozapine plasma levels. However, the different inverse agonistic potency at the 5HT2c receptor could explain this association (298-301).

Clozapine displays a high inter-individual variability in dose-corrected plasma concentrations which might be explained by the influence of genetic and environmental factors on the metabolism and drug transport of clozapine (112;302;303).

In vitro studies suggest the contribution of several Cytochrome P450 enzymes (CYP) in the metabolism of clozapine (181;186;304): CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A, with CYP1A2 having a major role in the N-demethylation of clozapine to its main active metabolite norclozapine (304). Figure 13 illustrates probable metabolic pathways of clozapine. In vivo the role of CYP1A2 has been confirmed (69;305), and it explains the lower plasma levels in smokers compared to non-smokers (306) since CYP1A2 is induced by smoking (307). A small pharmacogenetic study

did not find a significant influence of CYP2D6 nor CYP2C19 in the in vivo metabolism of clozapine (308). CYP3A could play a significant role since clozapine plasma concentration is lowered in presence of carbamazepine, a strong CYP3A inducer (309) but it is not known which CYP3A isoforms are implicated.

Figure 13: Possible metabolic pathways of clozapine (304;310-312)



Clozapine could be a substrate of the P-glycoprotein (Pgp), encoded by the *ABCB1* gene, as found in one in vitro study (313) but not found in another (314).

The aim of this study was to examine the in vivo influence of genetic polymorphisms of CYP isoforms (*CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP3A4, CYP3A5, and CYP3A7*) and *ABCB1* on the steady state plasma concentrations of clozapine. Patients were also phenotyped with the caffeine test for CYP1A2 and the midazolam test for CYP3A since genotyping reflects only partly the activity of these two CYP enzymes. Further environmental factors such as smoking and co-medication were investigated. Finally, we examined a possible causal association of clozapine plasma concentrations with side effects, especially with weight gain (315;316).

Methods

Seventy-five in-patients of 2 psychiatric clinics aged 18 years and older, on stable clozapine treatment and unchanged co-medication for at least 2 weeks (4 weeks for fluoxetine) were included in the study. Serious uncontrolled illnesses, organic psychiatric illness, or substance dependence were exclusion

criteria. To ensure compliance the patients took their medication under the supervision of a nurse, clozapine being dissolved in the four days before blood sampling. Patients or their legal representative signed the written informed consent, and the study was approved by the local ethics committees of the 2 clinics (Königsfelden and Prilly-Lausanne).

Blood sampling: on the morning of day 1, before first drug intake, 75µg oral midazolam diluted in a glass of water was given to the patient for CYP3A phenotyping (152). A blood sample was taken 30 minutes later for the determination of the 1'OH-midazolam/midazolam plasma ratio (152) and trough clozapine and norclozapine plasma concentrations. Thereafter the patients received their usual medication together with 200mg caffeine for CYP1A2 phenotyping (317). 6 hours later a second blood sample was taken for the determination of the paraxanthine/caffeine plasma ratio (317). No caffeine containing food or beverage was allowed on the test day until after the second blood sampling. All the samples – plasma after centrifugation and K-EDTA whole blood – were kept frozen at -20ºC until analysis. To control compliance, plasma concentration measurements of clozapine and norclozapine were repeated on day 7. Since there were no significant differences between them, results are expressed as the mean of the 2 blood samplings.

Assays of drugs: Clozapine and norclozapine concentrations were determined by gas chromatography with a nitrogen-phosphorus detector (69). Fluvoxamine (318), midazolam and 1′OH-midazolam (151;152) caffeine and paraxanthine (69) were measured by gas chromatography-mass spectrometry. Measured clozapine and norclozapine plasma concentrations were corrected by clozapine daily dose, and hereafter are referred to as plasma concentrations.

Genotyping: Genomic DNA was extracted from EDTA blood samples with the FlexiGene DNA Kit (Qiagen, Hombrechtikon, Switzerland). All the SNPs, with the exception of CYP2D6*5 and CYP2D6*xN, were detected by real-time PCR with 5'-nuclease allelic discrimination assays (ABI PRISM 7000 Sequence Detection System, Applied Biosystems, Rotkreuz, Switzerland) with primers and probes obtained from Applied Biosystems. The CYP1A2*1F, CYP2B6*4, CYP2B6*5, CYP2B6*6, CYP2B6*7, CYP2B6*9, CYP2C9*2, CYP2C9*3, CYP2C19*2, CYP2C19*3, CYP2D6*3, CYP2D6*4, CYP2D6*6, CYP3A4*1B, CYP3A5*3, ABCB1 61A>G, 2677G>T and 3435C>T SNPs were analyzed as previously described (69;138). CYP2D6 gene deletion (allele *5) and duplication/multi-duplication (allele *xN) were analyzed by quantitative real-time PCR and long PCR, respectively (138). CYP3A7*1C (-262T>A and -270T>G) allele was determined as previously described (319). CYP2C19*17 (-806C>T) allele was determined using the following primers, GTTTGGAAGTTGTTTTGTTTTGCTAA (forward), CATCGTGGCGCATTATCTCTT (reverse), and labelled probes, 6-FAM-TTCTCAAAGcATCTCT-MGBNFQ, and VIC–TTCTGTTCTCAAAGtATCT-MGBNFQ. The 25µl PCR mixture contained 12.5µl TaqMan Universal

PCR Master Mix (Applied Biosystems), 900nM of each primer, 200nM of each TaqMan minor groove binder non-fluorescent quencher probe, and 40ng (100ng for CYP2C19*17) of genomic DNA. After an activation step comprising AmpErase (50°C for 2 min) and AmpliTaq Gold enzyme activation (95°C for 10min), 60 PCR cycles (50 cycles for CYP2C19*17) were performed with 15s at 92°C and 1min at 58°C (1.5min at 60ºC for CYP2C19*17). CYP3A4 rs4646437C>T were analyzed with commercial TaqMan® Drug Metabolism Genotyping Assays according to the manufacturer's instructions (Assay Ids C_32306227_10; Applied Biosystems).

Clinical assessments: At baseline all patients underwent a physical examination and routine haematological and chemistry parameters were measured. Their medical history was recorded and their psychiatric and somatic diagnoses were confirmed. On days 1 and 7, weight, vital signs, adverse events and lifestyle factors such as smoking, caffeine consumption and grapefruit intake were noted. Weight gain data during the entire clozapine treatment were collected from the patient's medical files.

Statistical Analysis: Clozapine and norclozapine blood concentrations were compared between different genotypes by non-parametric analyses (Kruskal-Wallis for >2, Mann-Whitney U Test for 2 groups). Correlations between plasma concentrations and CYP1A2 or CYP3A activity were assessed by Spearman's test, and multivariate analyses were performed using linear regression (backward method). A p-value <0.05 was considered to indicate statistical significance. All statistical tests were performed in the whole group of patients and in the two subgroups with and without fluvoxamine as inhibition by fluvoxamine could mask the potential influence of other factors. Statistical analyses were performed using SPSS version 15.0 (SPSS Inc, Chicago, IL, USA). For ABCB1 polymorphisms, Hardy-Weinberg equilibrium was tested and linkage disequilibrium (Lewontin's D'coefficient) was estimated with STATA (version 10, StataCorp, College Station TX, USA). Haplotypes were inferred using the haplo.em function in R (http://www.r-project.org/), which uses expectation-maximization algorithm. As none of the inferred haplotypes had a posterior probability below 98%, haplotype uncertainty can be considered as minimal. Genetic association studies were conducted using the haplo.score function in R (which uses generalized linear models and takes haplotype uncertainty into account) with an additive effect and a Gaussian distribution for the trait.

Results

Seventy-five patients (39 men/36 women; 73 Caucasian/1 Asian/1 Black African) participated in the study. Their median age was 44 years (mean 48; SD 17; range 20-90). The majority were diagnosed with schizophrenic disorders (n=73), one with bipolar disorder, and one with dementia.

Table 19 gives an overview of the observed genotype frequencies of *CYP1A2*, *CYP2B6*, *CYP2C9*, *CYP2C19*, *CYP2D6*, CYP3A4, *CYP3A5*, *CYP3A7* and *ABCB1*. They are similar to those previously described in Caucasian populations (http://www.cypalleles.ki.se) (64;139)and all the SNPs are in

Hardy-Weinberg equilibrium for the Caucasian sub-sample (n=73). All 3 SNPs of the *ABCB1* genes are in strong linkage disequilibrium, as previously reported (136).

*Table 19: Frequency of CYP1A2*1F, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP3A4, CYP3A5, CYP3A7 and ABCB1 genotypes in 73 white patients treated with clozapine*

Genotype		**n**	**Frequency (%)**	**95% CI (%)**
	CYP1A2*1F			
	**1/*1*	8	10.9	4.8-20.5
	**1/*1F*	31	42.5	31.0-54.6
	**1F/*1F*	34	46.6	34.8-58.6
	CYP2B6			
	**1/*1*	30	41.1	29.7-53.2
	**1/*4*	1	1.4	0.03-7.4
	**1/*5*	8	10.9	4.8-20.5
	**1/*6*	20	27.4	17.6-39.1
	**1/*7*	4	5.5	1.5-13.4
	**5/*5*	2	2.7	0.3-9.5
	**6/*6*	8	10.9	4.8-20.5
	CYP2C9			
	**1/*1*	51	69.9	58.0-80.1
	**1/*2*	11	15.1	7.8-25.4
	**1/*3*	8	10.9	4.8-20.5
	**2/*2*	1	1.4	0.03-7.4
	**2/*3*	2	2.7	0.3-9.5
	CYP2C19			
	**1/*1*	24	32.9	22.3-44.9
	**1/*2*	17	23.3	14.2-34.6
	**1/*17*	18	24.6	15.3-36.1
	**2/*2*	4	5.5	1.5-13.4
	**2/*17*	4	5.5	1.5-13.4
	**17/*17*	6	8.2	3.1-17.0
	CYP2D6			
	**1/*1*	40	54.8	42.7-66.5
	**1/*3*	4	5.5	1.5-13.4
	**1/*4*	16	21.9	13.1-33.1
	**1/*5*	3	4.1	0.9-11.5
	**1/*6*	1	1.4	0.03-7.4
	**1/*xN*	4	5.5	1.5-13.4
	**4/*4*	4	5.5	1.5-13.4
	**4/*xN*	1	1.4	0.03-7.4
CYP3A	***CYP3A5*3***			
	**1/*1*	1	1.4	0.03-7.4
	**1/*3*	8	10.9	4.8-20.5
	**3/*3*	64	87.7	77.9-94.2
	CYP3A7*1C			
	**1/*1*	66	90.4	81.2-96.1
	**1/*1C*	6	8.2	3.1-17.0
	**1C*1C*	1	1.4	0.03-7.4
	CYP3A4 rs4646437 (intron 7)			
	CC	58	79.4	68.4-88.0
	CT	14	19.2	10.9-30.1
	TT	1	1.4	0.03-7.4
ABCB1	***61A>G (exon2)***			
	AA	71	97.3	90.5-99.7
	AG	2	2.7	0.3-9.5
	2677G>T (exon 21)			
	GG	25	34.2	23.5-46.3
	GT	38	52.1	40.0-63.9
	TT	10	13.7	6.8-23.8
	3435C>T (exon 26)			
	CC	18	24.6	15.3-36.1
	CT	40	54.8	42.7-66.5
	TT	15	20.5	12.0-31.6

Dose-plasma level relation

The median clozapine daily dose was 250mg (range: 25-800mg). Six patients received clozapine mono-therapy; 17 patients (23%) had co-medication with the strong CYP1A2 and moderate CYP3A and 2C19 inhibitor fluvoxamine (dose range: 25-300mg/day) (320;321). The dose corrected median trough plasma concentrations of clozapine and norclozapine were 1.14ng/ml*mg (0.15-6.24) and 0.60ng/ml*mg (0.04-2.36) in the whole group of patients and 0.99ng/ml*mg (0.15-2.88) and 0.49ng/ml*mg (0.04-1.28) in the group of patients without fluvoxamine, respectively. The median clozapine, norclozapine, and clozapine + norclozapine plasma concentrations were 3.5-, 2.4- and 3.3-fold higher, respectively, in the group with as compared to the group without fluvoxamine ($p=4.9 \times 10^{-7}$, $p=1.3 \times 10^{-5}$ and $p=1.1 \times 10^{-6}$, respectively). Correlations (logarithmic regressions) were calculated between fluvoxamine plasma concentrations and clozapine ($r^2=0.65$), norclozapine ($r^2=0.11$) and clozapine + norclozapine ($r^2=0.52$) plasma concentrations (fig. 14). In addition, this figure suggests saturation of inhibition in the range 50 to 100ng/ml of fluvoxamine. In agreement with a strong inhibition of CYP1A2 activity by fluvoxamine, the median paraxanthine/caffeine ratios were 0.72 (0.19–3.12) and 0.33 (0.08–3.49) in the groups of patients without and with fluvoxamine, respectively. Flattening of the correlation curve (power regression, $r^2=0.71$) between fluvoxamine plasma concentrations and paraxanthine/caffeine ratios suggests saturation of the inhibition of CYP1A2 activity with increasing fluvoxamine plasma concentrations (fig. 15).

Figure 14: Correlations (logarithmic regressions) between fluvoxamine plasma levels and (■) *clozapine (y=0.84Ln(x) +0.88, $r^2=0.65$),* (▲) *norclozapine (y=0.11Ln(x) +0.85, $r^2=0.11$) and* (○) *clozapine + norclozapine (y=0.96Ln(x) +1.73, $r^2=0.52$) dose normalised plasma levels.*

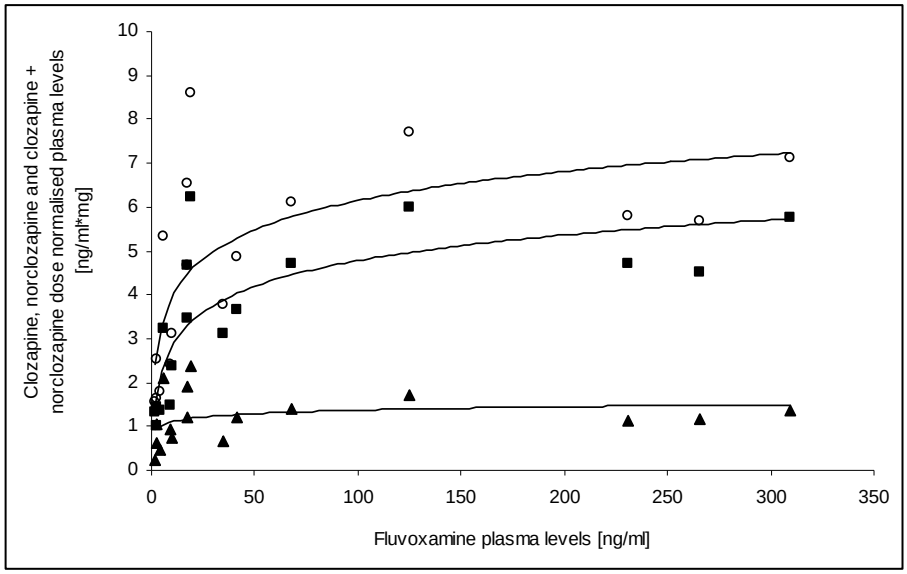
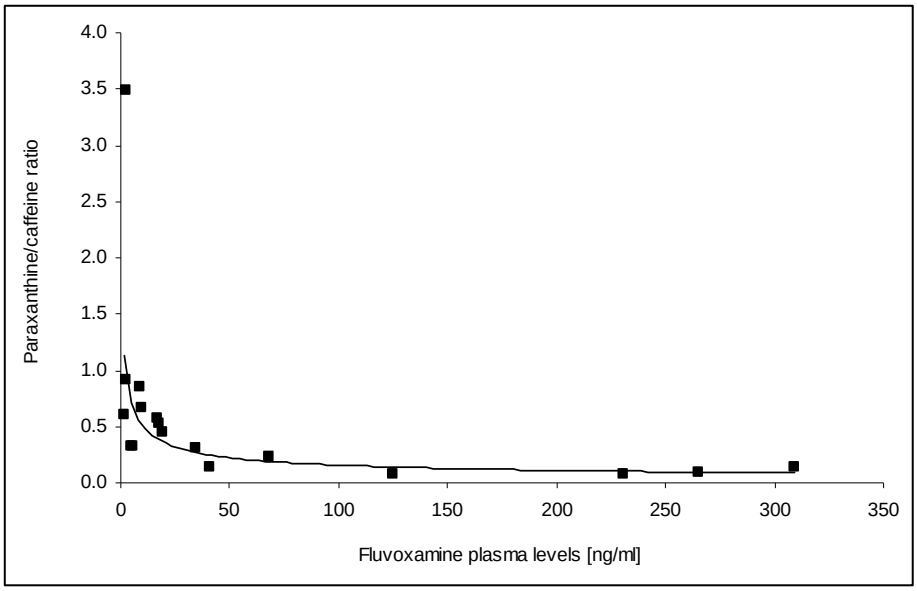


Figure 15: Correlation between fluvoxamine plasma levels and CYP1A2 activity measured by the paraxanthine/caffeine ratio (power regression: $y=1.62x^{-0.51}$, $r^2=0.71$). The outlier corresponds to a patient with a CYP2D6 ultra rapid metaboliser polymorphism with very low fluvoxamine plasma levels.



A group of patients was identified with other possibly relevant co-medications (maximal dose; number of patients): sertraline (322) (150mg/day; 6), paroxetine (323) (40mg/day; 3), fluoxetine (324) (20mg/day; 1), levomepromazine (325) (150mg/day; 3), amlodipine (10mg/day; 2), phenytoin (326) (300mg/day; 1) and omeprazole (327) (20mg/day; 1). There was no significant effect of these co-medications on clozapine ($p > 0.3$), norclozapine ($p \geq 0.9$) or clozapine + norclozapine ($p > 0.6$) concentrations when considered individually or as a group. Gender and age in the total study population did not appear to influence clozapine plasma concentrations (p=0.34, p=0.43 respectively, data not shown). However, when excluding patients taking fluvoxamine, women had significantly higher clozapine but not norclozapine (p=0.12, data not shown) plasma concentrations (median: 1.11 (0.18–2.88) ng/ml*mg versus 0.61 (0.15-2.72) ng/m*mg, in women and men respectively, p=0.027).

Forty-five patients were smokers (26 men/19 women) and 30 were non-smokers (13 men/17 women). The number of cigarettes smoked per day ranged from 1 to 60 (median 20). Smoking induces CYP1A2 as shown by the 1.5-fold higher median paraxanthine/caffeine ratio (p=0.031) in smokers (0.74 (0.08–3.49)) compared with non-smokers (0.50 (0.09–1.15)). Lower norclozapine (median of 0.49 versus 0.67 ng/ml*mg, p=0.039), but not clozapine (1.03 versus 1.30 ng/ml*mg, p=0.175), plasma concentrations were measured in smokers compared with non-smokers. As expected, this effect was more pronounced in the group without fluvoxamine, where the influence of smoking was also significant on clozapine plasma concentrations (median of 0.72 versus 1.21ng/ml*mg, in smokers

and non-smokers, respectively, p=0.011). The effect of smoking on clozapine or norclozapine plasma concentrations was not related to the number (>20, 11 to 20, 6 to 10, ≤5) of cigarettes smoked per day (data not shown).

Since only three patients drank grapefruit juice, and all but two had regular caffeine intake, the effect of grapefruit and caffeine on clozapine plasma concentrations could not be determined. In contrast to two previous studies (315;316), there was no significant correlation between norclozapine plasma levels (not corrected by dose) and weight gain (r=0.11, p=0.38), nor after subgroup analysis of non-smokers (r=0.28, p=0.14) and smokers (r=-0.07, p=0.65).

Plasma level and side effects

Clozapine treatment was in general well tolerated at the time of the study; the most frequent complaints were hyper-salivation and weight gain. Four patients developed diabetes in the course of their clozapine treatment, but all before this study.

The median weight at entry to the study was 79 kg (range: 52-128 kg; 74.5 kg and 83 kg, for women and men, respectively). The median body mass index (BMI) was 27.4 kg/m^2 (range: 19.1-36.6). Thirty-two (43%) patients gained 10% or more of their starting body weight during the course of clozapine treatment, with the maximum increase being 97% over 15 years for a male aged 32 with a BMI of 36.6 kg/m^2. Three patients lost weight, 25 remained stable, 13 increased their weight slightly to moderately (<10% of body weight), and for 2 patients the initial body weight was unknown.

In contrast to two previous studies (315;316), there was no significant correlation between norclozapine plasma levels (not corrected by dose) and weight gain (r=0.11, p=0.38), nor after subgroup analysis of non-smokers (r=0.28, p=0.14) and smokers (r=-0.07, p=0.65).

Hyper-salivation did correlate with neither clozapine nor norclozapine plasma levels.

Cytochrome 1A2:

*Induction polymorphism CYP1A2*F*

Genetic polymorphisms for the CYP1A2*F allele were without influence on clozapine, norclozapine or clozapine + norclozapine plasma levels: *CYP1A2* (p=0.386, 0.632, 0.533), in the whole group (and in the patients without fluvoxamine (data not shown)).

Phenotyping with the caffeine test

A strong correlation was observed between CYP1A2 activity and plasma concentrations of clozapine, norclozapine and clozapine + norclozapine in the whole population (r=-0.61, p=1·10^{-6}; r=-0.48,

p=2·10^{-5}; r=-0.59, p=1·10^{-6}), in the subgroup without fluvoxamine (n=58) (r=-0.51, p=5·10^{-5}; r=-0.41, p=0.001; r=-0.50, p=1·10^{-4}), and in the fluvoxamine subgroup (n=17) (r=-0.69, p=0.002; r=-0.39, p=0.12; r=-0.64, p=0.006).

Cytochrome 2C19 polymorphism

In the whole patient group (n=75) *CYP2C19* genotypes significantly influenced clozapine (p=0.036) but not norclozapine (p=0.185) plasma concentrations (Fig. 16a and b), with a 2.3-fold higher median clozapine concentrations in poor metabolisers (**2/*2* genotype, n=5, 2.58ng/ml*mg (1.10-5.98)) than in extensive metabolisers (non-*2/*2 genotypes, 1.11ng/ml*mg (0.15-6.24)) and 1.9-fold (p=0.057) higher clozapine + norclozapine levels. Similarly, between carriers of the *17 allele associated with an increased CYP2C19 activity (*17/*17, *1/*17) and poor metabolisers the differences were 2.3-, 1.9-, and 1.6-fold respectively for clozapine p=0.033, clozapine + norclozapine 0.039, and norclozapine 0.112. On the other hand, no significant differences in clozapine (p=0.558), norclozapine (p=0.186) and clozapine + norclozapine (p=0.407) plasma levels were found between the carriers of the *17 allele (*17/*17, *1/*17) and extensive metabolisers (*1/*1, *1/*2, *2/*17; data not shown). In the smaller group of patients without fluvoxamine, significant differences were observed between CYP2C19 *1/*1, *1/*17 or *17/*17 and *2/*17, *1/*2 or *2/*2 individuals for clozapine (p=0.027), norclozapine (p=0.074) and the sum of both (p=0.042).

Figure 16 a: Boxplot with median and interquartile range of clozapine plasma concentration (ng/ml x mg) according to CYP2C19 genotypes. (Patient nr. 38': clozapine plasma levels not detected).

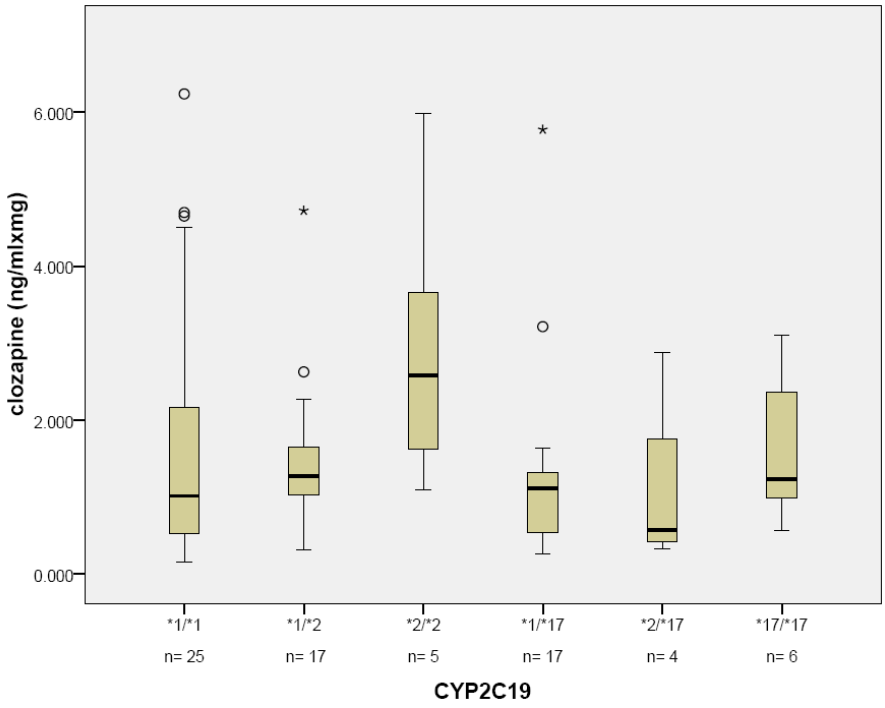
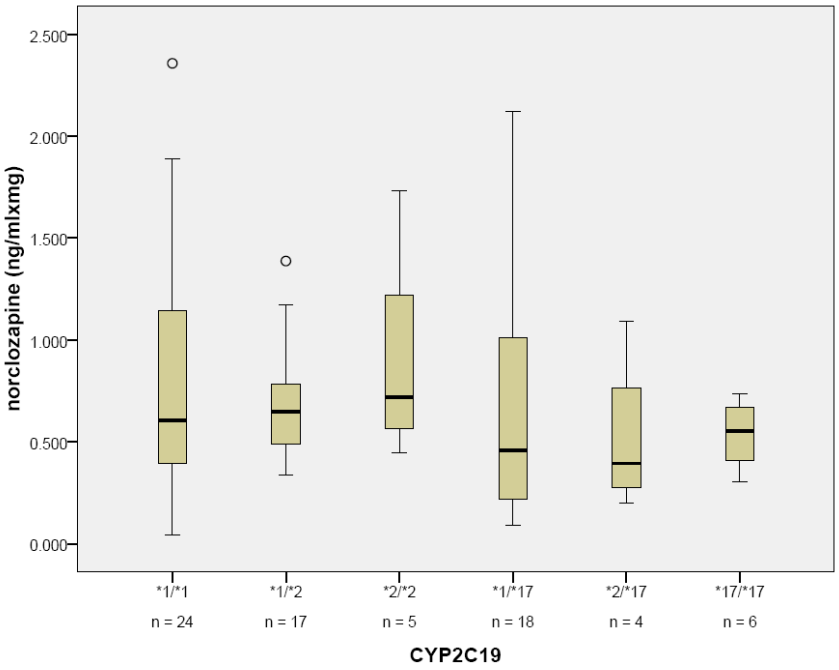


Figure 16 b: Boxplot with median and interquartile range of norclozapine plasma concentration (ng/ml x mg) according to CYP2C19 genotypes. (Patient nr.1': norclozapine plasma levels not detected)



Cytochrome 2D6 polymorphism

The CYP2D6 pharmacogenetic status of the patient had no influence on the plasma concentration of clozapine, norclozapine, and of the sum clozapine + norclozapine: (p=0.464, 0.696, and 0.718). The frequency of the different CYP2D6 genotypes was as expected in a white population (64).

Cytochrome 3A:

CYP 3A4, 3A5 and 3A7 polymorphisms

The CYP3A pharmacogenetic status of the patient was without influence on clozapine, norclozapine or clozapine + norclozapine plasma levels: *CYP3A4* (p=0.355, 0.341, 0.444), *CYP3A5* (p=0.865, 0.206, 0.627), *and CYP3A7* (p=0.586, 0.384, 0.493), in the whole group (and in the patients without fluvoxamine (data not shown)).

CYP3A phenotyping with the Midazolam Test

No correlation was found between clozapine (r=-0.16, p=0.16), norclozapine (r=-0.07, p=0.58), and clozapine + norclozapine (r=-0.161, p=0.172) plasma concentrations and CYP3A activity in the whole group. In the fluvoxamine subgroup, however, a weak correlation was found between CYP3A activity

and clozapine + norclozapine (r=0.51, p=0.038), a moderate correlation with norclozapine (r=0.63, p=0.007), and a trend with clozapine concentrations(r=0.44, p=0.075).

Other CYP450 isozyme polymorphism

Finally, other genetic polymorphisms were without influence on clozapine, norclozapine or clozapine + norclozapine plasma levels: *CYP2B6* (p=0.664, 0.540, 0.522), *CYP2C9* (p=0.252, 0.344, 0.370), in the whole group (and in the patients without fluvoxamine (data not shown)).

ABCB1 polymorphism

In the whole patient group (n=75) *ABCB1 3435 C>T* polymorphism significantly influenced clozapine plasma concentrations (p=0.046), with a 1.6-fold higher median clozapine concentrations in *3435TT* genotype (n=16, median=1.6 ng/ml*mg (0.27–5.98) in *TT* genotypes; n=59, median=1.1ng/ml*mg (0.15-6.24) in *CC/CT* genotypes). Statistical analysis on the *61 A>G* polymorphism was not performed due to the low observed genetic variability (table 22). No significant influence of the *2677 G>T* polymorphism on clozapine plasma concentration was observed (data not shown). In addition, norclozapine and clozapine + norclozapine plasma concentrations did not differ significantly between different genotypes (*2677G>T* and *3435C>T*) (data not shown). Haplotype analysis revealed a trend towards higher clozapine concentration for carriers of *2677G-3435T* haplotype (global score: 0.1, haplotype specific score: 0.01). Because of the small sample size when considering haplotypes, we also computed permutation tests (global empirical p-value: 0.10; haplotype-specific empirical p-value: 0.01), which are in very close agreement with the asymptotic p-values based on a chi-square distribution. Similar results were obtained after adjusting for sex and age (data not shown).

Multivariate Analysis

Multivariate analyses between clozapine, norclozapine and clozapine + norclozapine plasma concentrations and the main factors potentially influencing their kinetics yielded the following models in the whole group of patients. For clozapine, presence of fluvoxamine ($p<10^{-8}$), high fluvoxamine concentrations (p=0.0001), low CYP1A2 activity (p=0.0001) and absence of *CYP2C19 *17*17* or **17/*1* genotype (p=0.008) were predictive of higher plasma concentrations (r=0.84, $p<10^{-17}$). Other variables such as fluvoxamine dose (p=0.88), gender (p=0.19), smoking (p=0.29), CYP3A activity (p=0.67), *CYP3A4 rs4646437* allele T (p=0.69), *CYP1A2*1F/1F* genotype (p=0.32), *ABCB1* 2677TT genotype (p=0.22) and *ABCB1* 3435TT genotype (p=0.17) did not significantly contribute to the model. For norclozapine, presence of fluvoxamine ($p<10^{-8}$), non-smoking (p=0.004), low CYP1A2 activity (p=0.025) and absence of *CYP2C19 *17*17* or **17/*1* genotype (p=0.036) were predictive of higher plasma concentrations (r=0.72, $p<10^{-9}$). For clozapine + norclozapine, presence of fluvoxamine ($p<10^{-8}$), high fluvoxamine concentrations (p=0.004), low CYP1A2 activity (p=0.0001) and absence

of *CYP2C19 *17*17* or **17/*1* genotype (p=0.012) were predictive of higher plasma concentrations (r=0.82, $p<10^{-15}$). Similar models can be built including presence of *CYP2C19 *2/*2 or *2/*1* genotype instead of absence of *CYP2C19 *17*17 or *17/*1* genotype as a significant covariate for higher clozapine (p=0.017) and clozapine + norclozapine (p=0.030) plasma concentrations.

Discussion

The measured trough plasma concentrations of clozapine, norclozapine, and clozapine + norclozapine corrected by daily dose showed very wide inter-individual variability, with a 41-, 59-, 23-fold variation, respectively. Determination of genetic and environmental factors contributing to this variation is therefore of clinical relevance, considering the narrow therapeutic window of clozapine (350-600ng/ml) (287); plasma levels over 800-1000ng/ml are associated with an increased risk of side effects such as convulsions (289). Previous in vitro and in vivo studies suggested that the main CYP isoform mediating the metabolism of clozapine is CYP1A2 (112;186;305). Therefore, modulation of CYP1A2 activity will have a major influence on clozapine plasma levels and its effects. We examined 4 factors believed to have a relevant influence on CYP1A2 activity: *CYP1A2*1F* polymorphism, the effect of smoking and caffeine consumption, and co-medication with fluvoxamine.

*CYP1A2*1F* has been associated with increased CYP1A2 activity in smokers, possibly due to increased inducibility (69;70). Contrary to two previous studies (69;70), but in accordance with two other (71;279), we could not confirm an influence of *CYP1A2*1F* polymorphism on clozapine plasma concentrations or CYP1A2 activity, either in the whole group or in the subgroup of smokers; a strong influence of this polymorphism on clozapine plasma concentrations appears therefore unlikely. On the other hand, the important inducing effect of smoking on CYP1A2 activity and clozapine metabolism (306) has been confirmed in our study by the 1.5-fold higher CYP1A2 activity in smokers compared with non-smokers in all patients and in those without fluvoxamine as co-medication. Measured clozapine and norclozapine plasma levels in smokers compared with non-smokers were 93% (ns) and 77% (p=0.039) in the whole group, and 67% (p=0.011) and 64% (p=0.003) in the group without fluvoxamine. Interestingly the number of cigarettes seemed to be of little relevance. The demonstrated decrease in clozapine plasma concentrations in smokers is in accordance with most other studies (71;306;328;329). Considering the narrow therapeutic window of clozapine, therapeutic drug monitoring is recommended when smoking habits are changed, as cessation of smoking can lead to a significant rise in clozapine concentrations and the risk of overdose (212).

In the present study, 23% of the patients were co-medicated with the antidepressant fluvoxamine. Such high proportion is explained by the fact that in one study centre (Königsfelden) patients not responding and/or intolerant to high doses of clozapine are switched to a combination of low dose clozapine and fluvoxamine (– of course with therapeutic drug monitoring to adapt clozapine doses

(69;315;330)). Fluvoxamine is a strong CYP1A2 inhibitor, which is confirmed by the 2.2-fold higher paraxanthine/caffeine ratios determined in the patients without fluvoxamine compared to those with fluvoxamine. Accordingly, fluvoxamine markedly increases clozapine (3.5-fold) and norclozapine plasma concentrations (2.4-fold), indicating that it blocks the metabolism of both clozapine and norclozapine. The question arises whether the blocking effect of fluvoxamine on CYP1A2 is dose-dependent or is saturable at low doses. We investigated this in an earlier case series and the conclusion was that co-medication with 150mg/day fluvoxamine has the same blocking effect as 300mg/day (331). This is confirmed by the relationship between fluvoxamine, clozapine and norclozapine plasma concentrations (Fig.14) suggesting saturation of inhibition at low fluvoxamine plasma levels (around 50-100ng/ml). Thus, a daily dose of about 100mg fluvoxamine (41) would be sufficient to have a major blocking effect on the metabolic pathways of clozapine and norclozapine. Saturation of the inhibitory effect on CYP1A2 activity is also observed with paraxanthine/caffeine ratios at around 50ng/ml fluvoxamine (fig. 15). Finally, published studies have suggested that caffeine consumption, in particular when consumption fluctuates over time, can influence clozapine plasma concentrations, possibly by inhibition of CYP1A2 (219). In the present study, since all but two patients had regular intake of caffeine, the influence of caffeine on clozapine plasma concentrations could not be verified.

Conflicting results have been published on the implication and relative importance of other CYP isoforms besides CYP1A2 in the metabolism of clozapine and norclozapine (304;308;312;332). We found no evidence of an effect of CYP2B6, CY2C9, CYP2D6, CYP3A5, or CYP3A7 on the steady-state kinetics of clozapine or norclozapine. On the other hand, this seems to be the first study to demonstrate a significant in vivo involvement of CYP2C19 in the pharmacokinetics of clozapine, previously suggested by an in vitro study (304) but challenged by an in vivo study with a single oral low dose of clozapine (308). Thus, in the present study, *CYP2C19* poor metabolisers had 2.3-fold higher plasma concentrations of clozapine than patients with other *CYP2C19* genotypes (fig. 16a). The absence of a significant influence of the *CYP2C19*17* allele could be attributed to its limited effect especially when present in one copy only (60). A possible explanation for the negative results observed in the single dose (10 mg) study is that, with such a low oral dose (308), only CYP1A2 was responsible for the metabolism of clozapine.

Fluvoxamine is also a CYP2C19 inhibitor (320;333;334). One can hypothesise that only the metabolism of clozapine but not norclozapine is affected. That could explain the different impact of fluvoxamine metabolic inhibition on clozapine and norclozapine plasma levels (fig. 14).

The effect of CYP3A4 has been previously examined in interaction studies with CYP3A4 inhibitors and inducers (309;332). Based on in vitro data, it has been suggested that its role becomes increasingly relevant with higher doses of clozapine (304). In our study, the dose ranged from 25 to 800mg/day,

with a median of 250mg/day. In the whole study population, there was no correlation between CYP3A activity and clozapine or norclozapine plasma concentrations. On the other hand, the observed correlation between 1-OH-midazolam/midazolam ratios and clozapine plasma concentrations in the fluvoxamine co-medication group probably reflects the increasing importance of CYP3A4 in patients with blocked CYP1A2 activity. The formation of clozapine N-oxide is CYP3A4 dependent but this metabolite is less important than norclozapine (295) and its conversion back to clozapine is hypothesised (296;304).

The very strong inhibition of clozapine metabolism by fluvoxamine can be explained by the fact that fluvoxamine is not only a strong CYP1A2 inhibitor but also a moderate inhibitor of CYP3A4 (321;335) and CYP2C19 (320;333;334).

Finally, the present study was the first, to our knowledge, to suggest that clozapine plasma concentration is significantly influenced by the genetic polymorphism of the *ABCB1* gene, with higher concentrations measured in the *3435TT* genotype, a genotype previously associated with lower Pgp expression (136). The result of a clinical trial with 40 male and 20 female schizophrenic patients (published at the same time as our study) confirmed our findings (336). Interestingly, in their study, patients with the *ABCB1 3435CC* or *C/T* genotype needed higher clozapine daily doses than patients with the *3435TT* genotype in order to reach satisfactory therapeutic results. Since these *ABCB1* genotypes have an influence on clozapine plasma levels we can assume that Pgp plays a role in the intestinal absorption process of clozapine, regulating its bioavailability. A role of Pgp at the blood brain barrier has been evaluated earlier with negative results (314).

No serious adverse drug reactions were reported, but hyper-salivation and weight gain were frequently reported to be troublesome and difficult to manage. Sialorrhea is a consequence of the M1 agonistic properties of norclozapine, weight gain can be attributed to the H1 and 5HT2 inverse agonistic properties of both clozapine and norclozapine (table 18). Weight gain is considered one of the major side effects of clozapine and it is an important risk factor for developing a metabolic syndrome. In our study 43 percent of patients gained 10% or more body weight during clozapine treatment. Some authors found a reduced risk for weight gain when combining fluvoxamine with clozapine (315). Another group found a correlation between norclozapine plasma concentrations and weight gain in non-smoking patients (316). These results could not be confirmed in our study probably because of the small number of non-smokers included. Another limitation is that the duration of clozapine treatment and the nature of pre-treatment could not be determined for all patients and that some patients were co-medicated with valproic acid and lithium which are also associated with weight gain. Due to the important clinical problems associated with weight gain in patients treated with atypical antipsychotics (337), the role of norclozapine should be examined further in prospective longitudinal studies.

It would be interesting to compare the metabolic ratio of patients with a clozapine monotherapy with patients taking a combination of fluvoxamine and clozapine to search for differences in efficacy and tolerability as done by other authors (292;293).

In conclusion, our study examined thoroughly the in vivo implications of drug metabolizing enzymes and transporters in clozapine kinetics with the aim to explain its large inter-individual variability. CYP1A2 is the major CYP isoform involved in clozapine metabolism in vivo, with CYP2C19 contributing to a moderate extent and CYP3A4 contributing in the presence of co-medications that induce activity of this isozyme or when CYP1A2 is blocked by drugs such as fluvoxamine. *ABCB1* genetic polymorphism also contributes to clozapine pharmacokinetic variability. To our knowledge, this is the first study showing a significant in vivo role of CYP2C19 and the Pgp transporter in clozapine kinetics. Besides these genetic factors, environmental factors such as smoking or co-medications (e.g. fluvoxamine) markedly influence the kinetics of clozapine. Finally, because of the limited sample size, the results of the present study should be replicated by another study with a larger number of patients.

6 Nested case-control study in psychiatric in-patients: AMSP+

Summary

From experiences with individual cases we learned that high drug plasma concentration and certain CYP450 genotypes seem to be associated with an increased risk for (S)ADR. However, there exist no studies in psychiatry which confirm these risk factors for a patient population and which might justify routine TDM and/or routine pharmacogenetic testing.

We therefore performed a nested case-control study in the psychiatric in-patient clinic Königsfelden comprising 62 SADR cases and 82 matched controls in order to examine the feasibility of a nested matched control study design. Firstly, in an open cohort, SADR according to the AMSP criteria were collected and analyses of the plasma concentrations of suspected drugs made. In this phase of the AMSP+ project, correct TDM but also pharmacogenetic tests were introduced to the clinicians of the clinic. In a second phase 62 SADR cases were collected, their drug plasma levels analysed, a CYP2D6 genotyping and a midazolam test performed. These cases would be matched with 3 controls each, matching for the imputed drug (combination), gender and age group (< 65 or ≥ 65 years old).

Matching proved to be more difficult than expected and the original study design was changed to a non matched case-control study. Some of the reasons were the very heterogeneous patient population, drug combinations which were difficult to match because unusual or rare, and the difficult recruitment of controls.

Preliminary results showed that the group of SADR patients and the group of controls were similar in age, gender, medication (matching criteria) but also in weight, BMI, renal function and smoking behaviour. However, the odds ratio for drug plasma levels ≥ 120% of the upper reference limit was 3.49 CI95: 1.42-8.57 (p=0.005) in SADR patients compared to the control patients.

SADR patients had more often a CYP2D6 poor metaboliser genotype, controls more often a CYP2D6 ultra rapid genotype; however that was statistically no significant. Larger studies with more patients have to show if these results can be confirmed.

For future studies the difficulties of a nested matched control design has to be considered. A non-matched case-control study in a large cohort study seems more realistic and feasible. A multi-centre approach would help finding SADR cases and controls in a timelier manner.

Introduction

The usefulness of TDM and pharmacogenetic tests in single cases can be shown. However, larger clinical trials are needed to answer the question “in which situation or for which drug should we apply TDM and/or pharmacogenetic tests?” or “should we routinely measure plasma levels and/or perform pharmacogenetic tests in order to improve drug safety?”

Such large studies will in general exceed the possibilities of one study centre and are very cost intensive.

Fewer resources are needed for a nested case-control study which we performed within the dynamic AMSP cohort of the clinic Königsfelden: AMSP+. In a first phase TDM and Pharmacogenetic Tests were introduced in association with the causality assessment of serious adverse drug reactions. In the second phase a patient with a SADR (= case) was matched with control patients. The AMSP+ study must be seen as a feasibility study, because within this field of TDM and pharmacogenetics no previous case control studies have been carried out. Ethics approval from the local ethics committee has been obtained for both phases. Two questions were addressed in the AMSP+ study: 1) Do patients with a SADR have higher drug plasma levels? 2) Are certain CYP450 enzyme genotypes associated with an increased ADR risk?

Do ADR patients have higher drug plasma levels?

The majority of ADR are of type A, i.e. mostly drug concentration dependent. Better than the administered dose, drug plasma levels reflect the concentration of the drug at target site, in psychiatry mostly the brain. Although we know that in an individual patient the ADR risk with increasing drug plasma level will increase, clinical experience shows us that the threshold for developing an ADR is different between patients and that several other factors play a significant role as well. Establishing a therapeutic index of drug plasma levels is not for every drug possible. The question remains: do patients with an ADR have higher drug plasma levels?

Are certain CYP450 enzyme genotypes associated with an increased ADR risk?

Some authors found an increased risk in small clinical studies (see chapter “Pharmacogenetic Studies in Pharmacovigilance”). Interestingly, certain CYP450 enzyme genotypes predicting a decreased enzyme function seemed to show an increased ADR risk, but a connection with increased drug plasma concentrations was not always shown. A large case-control study might be an adequate study design to further examine these relationships.

Collaborations

For the pharmacovigilance parts of the AMSP+ study a close collaboration with the leaders of the AMSP project: Dr. Renate Grohmann, Prof. Rolf Engel (both university of Munich), Prof. Eckart

Rüther (university of Göttingen), Prof. Waldemar Greil (university Munich and Sanatorium Kilchberg), and Dr. Andreas Horvath (Sanatorium Kilchberg) were important.

Drug plasma levels have been analysed in the laboratories of Prof. Pierre Baumann and Prof. Dr. Chin Eap from the University of Lausanne and of Prof. Dr. Katharina Rentsch from the University of Zurich, both laboratories granting research prices for the analyses.

Pharmacogenetic tests have been done in the laboratory of Prof. Pierre Baumann and Prof. Dr. Chin Eap at the University of Lausanne, within the common research project free of charge.

Advice on the study design and methodology has been given by Prof. Richard Farmer, University of Surrey, Prof. Sammy Suissa, University of Montreal, and by Jan-Willem van der Velden, MD, all pharmacoepidemiologists and experts in Drug Safety studies. Literature searches and discussions with these three experts made clear that before initiating any large case-control study, we need feasibility studies.

Method: Feasibility study

Phase I Awareness - collection of SADR and plasma level analysis of imputed drugs

In a first phase, serious ADR cases from the clinic Königsfelden were collected within the AMSP project and blood samples for plasma level analysis of the medication involved were taken. Pharmacogenetic testing was only performed in cases where the presence of a particular pharmacogenetic status was suspected. This first phase was necessary to introduce the notion of TDM and pharmacogenetic testing in relation to the causality assessment of serious ADR in the daily clinical routine and to obtain the cooperation of the clinicians.

Phase II nested matched control study

In the second phase the aim was to test the feasibility of using a nested case-control study to investigate possible associations between drug plasma concentrations and/or CYP2D6 genotypes and the event of a serious adverse drug reaction.

For this purpose 1 SADR case was matched with 3 control patients for the same imputed drug (combination), gender and age group (< 65 or ≥ 65 years old). Plasma levels of the imputed drugs were analysed and CYP2D6 genotyping and CYP3A phenotyping were routinely performed in all cases.

Stimulated spontaneous ADE reporting

Systematic ADE reporting where every ADE is captured is very resource intense and only possible within a delimited research project. Stimulated spontaneous ADE reporting is also feasible in the context of the naturalistic setting of a large psychiatric hospital. The stimulation consisted in explaining the research and quality assurance project AMSP+ to all parties involved: in the first line to the clinicians, but also the nurses and the laboratory personnel. This procedure was repeated several times per year, combining it with AMSP case presentations. Furthermore, posters with the AMSP criteria for SADR and what to do in case such an SADR is found were posted in every ward office, easy to fill in forms were distributed to all clinicians and the wards to announce such cases to the AMSP drug monitors. In order to further motivate the clinicians, all AMSP cases were thoroughly documented for them and for the patient history.

Collection of SADR according to the AMSP definition

The AMSP+ study was performed in close collaboration with the clinicians of the 400 beds psychiatric in-patient hospital Königsfelden in Switzerland, medically directed by Dr.med. Mario Etzensberger (till end of 2008 and time of data collection), and member of the AMSP project since 2001. Three medical doctors (Patrik L. Stephan most of the cases, Lukas Ritz, and Evelyne Rechsteiner) acted as AMSP drug monitors coordinating the collection of the SADR, and making the first causality assessment after seeing the patient themselves and after taking the relevant patient history and further development of the ADR into consideration. This causality assessment was discussed with the treating clinicians and the author of the thesis before entering them in the central AMSP database at the University of Munich. There all entries were controlled for completeness of the case, inconsistencies and plausibility. Finally the cases were discussed at one of the Swiss case conferences with the other participants of the Swiss AMSP project (currently 13 psychiatric inpatient clinics, 13 manufacturers of psychotropic drugs, and members of the regional pharmacovigilance centres). If during this conference the causality was assessed differently, changes were added in the central database. Difficult cases were further discussed in one of the international conferences which participants of the different countries attend (mainly Germany, Austria and Switzerland). Again, if amendments to the cases are made, these are entered in the central database. All case reports including all amendments were also sent to the pharmacovigilance authorities and to the relevant pharmaceutical company.

Blood sampling for drug plasma level analysis

Blood samples were taken in the morning before any drug intake in order to obtain drug trough plasma concentrations, knowing that the drug free interval would vary between about 12 to 24 hours (for twice respectively once daily administration, evening respectively morning intake).

Blood samples were immediately centrifuged and serum was sent in the morning per priority mail to the laboratory. Blood samples obtained during the weekend were kept in the refrigerator till Monday morning when they were treated as described before.

All the samples were analysed under routine conditions when received and the results were sent back to the clinician. This procedure allowed the clinician to adjust their treatment and reflects a naturalistic study design.

Pharmacogenetic testing

Pharmacogenetic testing was not readily accepted by the clinicians in the beginning of the study. In phase I pharmacogenetic testing was only done in case of strong suspicion of an unusual genotype.

In Phase II every ADR patient and all matched control patients were genotyped for CYP2D6 (138), alleles *1,*3, *4, *5 and 2XN and wildtype, and phenotyped for CYP3A with the midazolam test (151;152;338). The results were sent to the clinician as soon as available, again in order to be of clinical help. This clearly was a motivation for them to collaborate in the study.

For every pharmacogenetic test, for research purpose or for diagnostic reasons, a written informed consent by the concerned person or his or her tutor has to be obtained. Not only for doctors not familiar with this kind of tests can it be challenging to explain to psychiatric ill patients what a pharmacogenetic test means for them. Further to the informed consent the treating or research physician has to explain the results of the test to the patient, unless he or she explicitly refuses this knowledge. For every patient the pharmacogenetic test results were interpreted for the treating clinicians in the test report, put in the patient history and a personal genetic pass in credit card format was given to the patient explaining very briefly the test results as well (fig. 17).

Figure 17: Example of short genetic information given to the patient in credit card format

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Ausweis Pharmakogenetik

Anna Muster

11.11.1941

CYP2D6 Genotyp *4/*4 = keine Aktivität

CYP2C19 Genotyp*1/*1= unauffällig (untersucht Allele *2, *3)

Therapie-Empfehlung:
Medikamente, die v.a. über CYP2D6 abgebaut werden vorsichtig dosieren. Grosszügige Indikation für Kontrolle von Plasmaspiegel, v.a. bei enger therapeutischer Breite und Nebenwirkungen.

16.2.2006

Matching criteria for the control group

Three patients of the dynamic cohort of the patient population of the clinic Königsfelden, without an ADR at the time of selection, were matched for gender, age group (< 65 or ≥ 65 years old), and imputed drug (combination) of the SADR case. Concerning the drug treatment they had to have the same drug(s) but were allowed to have other drugs besides as well. We did not match for the duration of drug intake with exception of the cases of weight gain where the controls had to have at least a month drug intake.

Collection of control patients

Ideally controls are selected shortly after the case is identified. Although lists with matching criteria were distributed to all wards, this did not always work. On one hand there were not enough patients fulfilling all criteria and on the other hand not all potential controls gave informed consent. Therefore we tried to form a pool of potential control patients to be matched with later cases. Regular meetings were arranged in the wards explaining the research project and recruiting control patients.

Statistical analysis

This feasibility study is primarily analysed descriptively. Odds ratios have been calculated between cases and controls (see also table 2).

Preliminary Results

From 62 collected SADR cases only 20 could be matched with 3 controls, 8 cases with 2 controls, 7 with 1 control, and 27 cases could not be matched at all. 5 SADR cases were 65 or more years old. None of them could be matched with control patients.

Patient characteristics AMSP+ phase II

37 (60%) SADR cases were female, 25 (40%) were male, mean age was 44 ±11 years, median 44 (20-80) years. 31 (50%) were smokers, 23 (37%) non smokers, and in the remaining 7 cases smoking behaviour is unknown.

All patients in the clinic Königsfelden are diagnosed according to the ICD-10 International Classification of Diseases. 29 (46%) patients suffered from a schizophrenic disorder (ICD10 - F2), 14 (23%) from an affective disorder (F3), 10 had a neurotic disorder (ICD10-F4), 4 a personality disorder (ICD10-F6), 3 a substance abuse disorder (ICD10-F1), and 2 an organic mental disorder (ICD10-F0).

Type of SADR were: 9 weight gain, 4 each for serotonin toxicity, exanthema, 3 each for somnolence, liver enzyme increase, hyponatremia, hair loss, 2 each for tachycardia, seizures, oedema, hypotension,

hypersalivation, extrapyramidal syndrome, delirium, and 1 each for vomiting, urinary retention, tremor, taste distortion, tardive dyskinesia, singultus, priapism, pericarditis, night mares, neutropenia, metabolic syndrome, intoxication due to a drug interaction, ileus, galactorrhoea, eosinophilia, dizziness, diarrhoea, bleeding, amaurosis.

Weight and body mass index (BMI) were: mean weight was 75 ± 15kg, median weight 72 (47-128); mean BMI 26.1 ± 4.6, median BMI 24.4 (17.2-38.1).

For the measure of the renal function, creatinine clearance was calculated: mean clearance was 113 ± 28 ml/min; median was 116 (40-197) ml/min.

Results of the CYP3A activity or midazolam test (ratio 1'OH midazolam/midazolam) were: for 50 patients results from the midazolam were obtained with a mean of 6.8 ± 5.7, median of 3.6 (0.28-68.4); as reference: After oral administration of 75 µg midazolam, the 30-min total 1'OH-midazolam/midazolam ratios measured without co-medication, with ketoconazole (a strong CYP3A-inhibitor) and with rifampicin (a strong CYP3A-inducer) were (mean ± SD): 6.23 ± 2.61, 0.79 ± 0.39 and 56.1 ± 12.4, respectively (152).

CYP2D6 genotype frequencies were as follows: 42 EM (68%), 14 IM (23%), 4 PM (6.5%), 1UM (1.6%), and 1 unknown. The PM had the following SADR and imputed medication:

1) Liver enzyme increase, tachycardia, hypotension, dizziness under clomipramine 300mg/d (plasma level of clomipramine + desmethyl clomipramine: 1228ng/ml; ref: 175-450ng/ml) and quetiapine 700mg/d (plasma level: 826ng/ml; ref. 70-300ng/ml). This same patient had also a low CYP3A activity in the midazolam test with a value of 1.1.

2) Metabolic syndrome under olanzapine 30mg/d (99ng/ml; ref: 20-80ng/ml) and valproate 1500mg/d (66ng/ml; ref: 50-100ng/ml), (additionally the antihypertensive combination atenolol/chlortalidone 50mg/ 12.5mg/d). The result of the midazolam test was 2.56.

3) Abnormal bleeding under acetyl salicylic acid 100mg/d (no plasma levels) and fluoxetine 40mg/d (total 1054ng/ml, ref. 120-300ng/ml; fluoxetine 667 ng/ml ; norfluoxetine 386ng/ml), (additionally olanzapine 10mg/d (plasma level 15ng/ml, ref. 20-80ng/ml), enalapril 2.5mg/d, torasemide 20mg/d, potassium, vitamin D). The result of the midazolam test was 4.06.

4) Hair loss under lithium (0.78 mmol/l, ref. 0.5-1.2 mmol/l), venlafaxine (total 206ng/ml; ref. 195-400ng/ml; venlafaxine 111ng/ml; O-desmethylvenlafaxine 95ng/ml). No result of the midazolam test was available.

35 drugs were imputed in the SADR cases (table 20).

Table 20: Listing of the imputed drugs of the SADR cases (several drugs per case possible). n.d. = non detected; nc = non compliant

drug	Imputed with SADR	SADR only attributed to this drug	Reference plasma levels in ng/ml (including active metabolites, other units mentioned)	Cases with high plasma levels (% of recommended upper limit)	Cases with low plasma levels (% of recommended lower limit)	Plasma level missing
Acetyl salicylic acid	1	0	no ref value	-	-	1
Alprazolam	1	0	20 - 40	-	n.d.	-
Aripiprazol	2	1	150 - 250	118, 120	-	-
Biperiden	3	0	no ref value	-	n.d.	2
Buspirone	1	0	1 – 5	-	-	1
Carbamazepine	4	3	4-10 µg/ml	113, 120, 125	-	1
Chlorprothixene	1	0	20 – 200	-	-	-
Citalopram	1	1	30 – 130	-	-	-
Clomipramine	1	0	175 – 450	273	-	-
Clotiapine	2	0	no ref value	no ref value	no ref value	1
Clozapine	13	8	350 – 600	109, 111 257,120, 186	14 (nc), 57, 66	-
Diclofenac	1	0	No ref value	-	-	1
Fluoxetine	1	0	120 – 300	351	-	-
Flupentixol	1	1	2 – 15	127	-	-
Fluvoxamine	5	2	150 - 300	494, 284	8 (nc)	-
Haloperidol	4	0	5 – 17	-	68, 64, 68, 33	-
Lamotrigine	1	0	3 -14 µg/ml	-		-
Levomepromazine	1	0	15 – 60	-		-
Lithium	4	1	0,5-1,2 mmol/l	139	-	-
Lorazepam	1	0	10 – 15	-	-	1
Methadone	2	0	400 – 600	-	31	1
Mianserin	1	0	15 – 70	204		-
Mirtazapine	2	1	30 – 80	-		1
Olanzapine	4	0	20 – 80	124	20, 97	-
Oxcarbazepine	2	0	10-35 µg/ml	-	-	-
Paroxetine	2	0	30 – 120	-	-	-

Promazine	2	0	no ref value	no ref value	no ref value	1
Quetiapine	5	2	70 – 300	275	-	1
Risperidone	2	2	20 – 60	-	-	-
Sertraline	2	1	10 – 50	-	-	-
Trazodone	1	1	700 – 1000	-	57	-
Valproate	13	2	50-100 µg/l	-	28, 86, 44	-
Venlafaxine	5	1	195 – 400	135, 571, 237	-	-
Zolpidem	1	0	80 – 150	-	69	-
Zuclopenthixol	6	2	4 – 50	182	-	1

99 times a drug has been imputed in our SADR cases, in 13 cases drug plasma levels are missing; from the rest (n=86): 23 (27%) had levels above the upper limit of the TDM reference level (41), 19 (22%) had drug plasma levels of 120% or more of this upper limit. 18 (21%) had drug plasma levels below the lower limit of the TDM reference level, 16 (19%) had drug plasma levels of 80% or less of this lower limit.

43 of the imputed drugs were antipsychotics, 24 were mood stabilising drugs, 21 antidepressants, 4 anxiolytics/hypnotics, 3 anticholinergic drugs, 2 analgesics and 2 methadone.

Control group characteristics

82 control patients could be matched to the 62 SADR cases, 47 (57%) females and 35 males. Mean age was 42 ± 9 years, median 44 (20-64) years. 46 (56%) were smokers, 34 were not smoking, and from 2 the smoking data are missing.

44 (53%) suffered from a schizophrenic disorder (ICD10-F2), 21 (26%) from an affective disorder (ICD10-F3), 8 had a substance abuse disorder (ICD10-F1), 7 a neurotic disorder (ICD10-F4, and 1 each had an organic disorder (ICD-10-F0), a behavioural disorder (ICD10-) F5, and a disorder of the psychological development (ICD10-F8).

Mean weight was 75 ± 13kg, median 72 (50 – 127) kg. Mean BMI was 26.1 ± 4.1, median 25.3 (18.5-39.5).

Mean creatinine clearance was 117 ± 29 ml/min, median was 112 (55-220) ml/min.

For 78 out of the 82 controls results from the midazolam test were available. Mean was 8.9 ± 6.8, median 5.4 (0.25-69.6).

The *CYP2D6* genotype frequencies were: 51 EM (62%), 25 IM (30%), 2 PM (2.4%), 4 UM (4.9%), and 1 unknown. The two PM had the following medication:

1) Trazodone with plasma levels of 283 ng/ml (40% of the lower reference limit)

2) Clozapine with 595 ng/ml (within the recommended reference plasma levels)

Table 21: Drug levels analysed in the control patients

drug	Number of plasma levels	Reference plasma levels in ng/ml (including active metabolites, other units mentioned)	Reference plasma levels	Controls with high plasma levels (% of recommended upper limit)	Controls with low plasma levels (% of recommended lower limit)	Plasma level missing
Acetyl salicylic acid	-	no ref value	no ref value	-	-	-
Alprazolam	-	20 - 40	20 - 40	-	-	-
Aripiprazol	6	150 - 250	150 - 250	160	45, 77	-
Biperiden	2	no ref value	no ref value	-	-	-
Buspirone	-	1 – 5	1 – 5	-	-	-
Carbamazepine	1	4-10 µg/ml	4-10 µg/ml	113	-	-
Chlorprothixene	-	20 – 200	20 – 200	-	-	-
Citalopram	3	30 – 130	30 – 130	-	-	-
Clomipramine + metabolite	-	175 – 450	175 – 450	-	-	-
Clotiapine	-	no ref value	no ref value	-	-	-
Clozapine	19	350 – 600	350 – 600	114, 137, 155	50, 90, 65, 65, 31, 17	-
Diclofenac	-	No ref value	No ref value	-	-	-
Fluoxetine	-	120 – 300	120 – 300	-	-	-
Flupentixol	-	2 – 15	2 – 15	-	-	-
Fluvoxamine	9	150 - 300	150 - 230	182, 207, 193	85, 90, 82, 47	-
Haloperidol	1	5 – 17	5 – 17	199	-	-
Lamotrigine	-	3 -14 µg/ml	3 -14 µg/ml	-	-	-
Levomepromazine	-	15 – 60	15 – 60	-	-	-
Lithium	5	0,5-1,2 mmol/l	0,5- 1,2 mmol/l	-	-	1

Lorazepam	-	10 – 15	10 – 15	-	-	-
Methadone	2	400 – 600	400 – 800	-	14, 89	-
Mianserin	3	15 – 70	15 – 70	180	86	-
Mirtazapine	3	30 – 80	40 – 80	-	96, 66	-
Olanzapine	5	20 – 80	20 – 80	-	88	-
Oxcarbazepine	-	10-35 µg/ml	10-35 µg/ml	-	-	-
Paroxetine	-	30 – 120	70 – 120	-	-	-
Promazine	-	no ref value	no ref value	-	-	-
Quetiapine	9	70 – 300	70 – 170	107	74, 74, 46, 87	-
Risperidone + metabolite	6	20 – 60	20 – 60	-	55, 70, 35, 45	-
Sertraline	2	10 – 50	10 – 50	-	90	-
Trazodone	3	700 – 1000	650 – 1500	-	44, 40	-
Valproate	21	50-100 µg/l	50-100 µg/l	105, 110, 115, 111	20, 92, 82	2
Venlafaxine + metabolite	5	195 – 400	195 – 400	186, 117	96	-
Zolpidem	-	80 – 150	80 – 150	-	-	-
Zuclopenthixol	6	4 – 50	4 – 50	-	-	-

108 plasma levels of control patients have been analysed (table 21). 17 (16%) were higher than the upper limit of the TDM reference value, 9 (8%) were 120% or higher. 33 (31%) controls had lower plasma concentrations than the lower limit of the TDM reference, 20 (19%) were 80% or lower.

Comparison of cases and control

Considering the low number of matched case-control groups we abandoned the analysis of a matched control study and compared all cases with all controls which led to the following results:

Cases and controls are similar for gender, age, weight, BMI, renal function, and smoking behaviour. The diagnose frequencies differ somewhat; in the control group the two main diagnosis groups F2 plus F3 sum up to 80%, in the cases to70%; more control patients suffered from an F1, and more cases from an F6 diagnosis.

A preliminary analysis of the data suggests that the CYP3A activity as measured by the midazolam test seems somewhat lower in the cases (mean 6.8 ± 5.7, median of 3.6 (0.28-68.4)) than controls (mean 8.9 ± 6.8, median 5.4 (0.25-69.6)).

The two groups seem also to differ in *CYP2D6* genotype frequencies with an odds ratio of 2.84 CI95, calculated according to the method explained in table 2: 0.58-13.69 (p=0.20) for PM, and for UM 0.33 CI95: 0.05-2.3 (p=0.3) but this does not reach statistical significance.

In the cases the 4 PM were under treatment with drugs being at least partly metabolised by CYP2D6: clomipramine, olanzapine, fluoxetine and venlafaxine. The 2 PM of the controls did get drugs which are not metabolised by CYP2D6: trazodone and clozapine.

Drug plasma concentrations of the cases compared to those of the controls seem to be more often higher than the upper reference level. The odds ratio for high plasma levels is: 1.95 CI95: 0.97-3.92 (p=0.045), restricting the analysis to plasma levels of 120% or more the odds ratio reaches 3.49 CI95: 1.42-8.57 (p=0.005).

Comparing the number of cases (n=17) with that of the controls (n=8) having drug levels higher than 120% of the upper reference level, the odds ratio is 3.11 CI95: 1.35-7.17 (p=0.006).

Discussion

This first analysis of the results of this study shows some positive but also negative aspects which hindered the full realisation of all the aims. To select matched control patients turned out to be much more difficult than anticipated. There was the problem of obtaining written informed consent, which is recognized to be a challenge in psychiatry since the first obligation is to evaluate a mentally ill patient's competence to consent (339). In the case of psychotic patients, they were not able to give informed consent, and often no tutor was appointed. In cases where the patient was able to give informed consent or where a tutor was available, the challenge of explaining the reasons for pharmacogenetic tests had to be met. Another hurdle was the phenotyping with the midazolam test. In our experience, psychiatric patients are not easily willing to drink test substances like the midazolam solution. Further many potential control patients were very ill, and they were not willing to consent to in their opinion unnecessary procedures such as taking extra blood samples for tests they were not interested in.

A study in a naturalistic setting of a large psychiatric inpatient clinic seems on first sight easier to perform than clinical trials with stringent in- and exclusion criteria. However, that is not necessarily true. First we are confronted with a broad heterogeneity of the patient population, with many different

diagnoses, drug treatments and co-morbidities. In-patients of such a clinic are very ill and their medication is mostly complex.

When an SADR occurs, they naturally tend to occur in patients with heavy drug treatments or with drugs with a less favourable side effect profile. Often these patients do not respond to other better tolerated medication and represent a special patient selection (selection bias). Matching control patients with the same medication proved sometimes impossible. In case of carbamazepine treatment this was prominent.

Some ADR such as heavy weight gain were common under certain medications such us olanzapine or clozapine (in line with the literature (340-342), matching these cases was not easy because the ADR is so common with these drugs, but also because of the time component of gaining weight.

Some drug combinations were so rare (e.g. haloperidol and clozapine) that we could not find matched controls, amongst other combinations with interaction risks such diclofenac with lithium. However, in some cases it might be acceptable to match only for the plasma concentration of the SADR causative agent and not for the interacting substance as well. No comparable pharmacovigilance study could be found where ADR cases have been compared in a matched control design with control patients under the same medication (combination) but without the ADR.

Another problem is the latency between the moments a SADR appears and the plasma concentrations are measured. Side effects are a major factor leading to non-compliance, and there are some cases where we suspect at least partial mal-compliance at the moment the blood sampling was taken. Therefore the number of patients with a high drug plasma concentration may be underestimated.

Comparison of the cases and controls showed similar characteristics. However, for the targeted risk factors such as high plasma levels or *CYP2D6 genotypes* associated with low CYP2D6 activity differences appeared. CYP2D6 PM are expected to have higher plasma levels of drugs being substrate of CYP2D6 and therefore might be more vulnerable to concentration dependent side effects. In a retrospective matched case control study (343) 18 CP2D6 PM were matched each with one CYP2D6 IM and on CYP2D6 EM. The risk for extrapyramidal symptoms or tardive dyskinesia, and for mal-compliance, was significantly higher in CYP2D6 poor metaboliser than in patients with another CYP2D6 genotype. Another study showed that the CYP2D6 genotype had a significant and clinically relevant influence on risperidone plasma concentrations and that average plasma concentration of the active moiety of risperidone was significantly higher in persons suffering from dystonia or parkinsonism (344). In our population the 4 SADR cases with a CYP2D6 poor metaboliser genotype were all under medication (clomipramine, olanzapine, fluoxetine, venlafaxine) which was at least partly metabolised by CYP2D6, 3 had higher than recommended plasma levels of these drugs, the

patient under venlafaxine treatment at the time of the blood sampling not. This could be due to poor compliance as consequence of intolerability. In contrast to the 4 cases the 2 CYP2D6 PM control patients were not treated with a medication metabolised by CYP2D6 (trazodone, clozapine) and their drug levels were not exceeding the recommended plasma concentrations. Although the odds ratios for *CYP2D6* genotype did not reach statistical significance, CYP2D6 PM seem to have a higher SADR risk, while CYP2D6 UM seem to have a protective effect. One can expect that with a higher number of patients and controls these odds ratios become statistically significant. In order to estimate the importance of certain genetic factors in drug safety, more and larger prospective studies in drug development and post marketing must examine this potential risk (345).

A recent review (346) summarises the results of studies on the effect of certain genotype on therapeutic efficacy and side effects of antipsychotics. Most of these were case-control or cohort but not matched control studies. CYP2D6 poor metaboliser showed in the majority a higher risk for extrapyramidal side effects (EPS); for tardive dyskinesia this relation was less clear. The studies showing a significant CYP2D6 genotype dependent risk for EPS/tardive dyskinesia comprised 50 or more cases.

Up to today we do not know the number of patients to genotype to prevent a serious side effect, a prerequisite for routine testing. And we do not yet have sufficient data to support or reject genotype based dosing as proposed by Kirchheiner et al (154;156;238).

High plasma levels (120% or more of the upper reference level) could be identified as a risk factor, in analysing the number of patients/controls with high plasma concentrations or in comparing the number of high plasma levels between the two groups. Other groups found also a higher risk with higher plasma levels with certain antipsychotics such as risperidone (344), clozapine (289) or tricyclic antidepressants (37).

In our feasibility study we had not enough matched case-control-pairs so that an unmatched case-control analysis has been performed and odds ratios were calculated. With our limited number of cases and controls this showed statistically significant results for high plasma levels and a trend for *CYP2D6* polymorphisms. A more extensive analysis about the influence of high plasma levels will be performed at a later stage adding more SADR cases to the current study cohort including plasma concentration information but without genotype determination and using more (unmatched) control patients (patients without an SADR).

Heterogeneity of the cohort

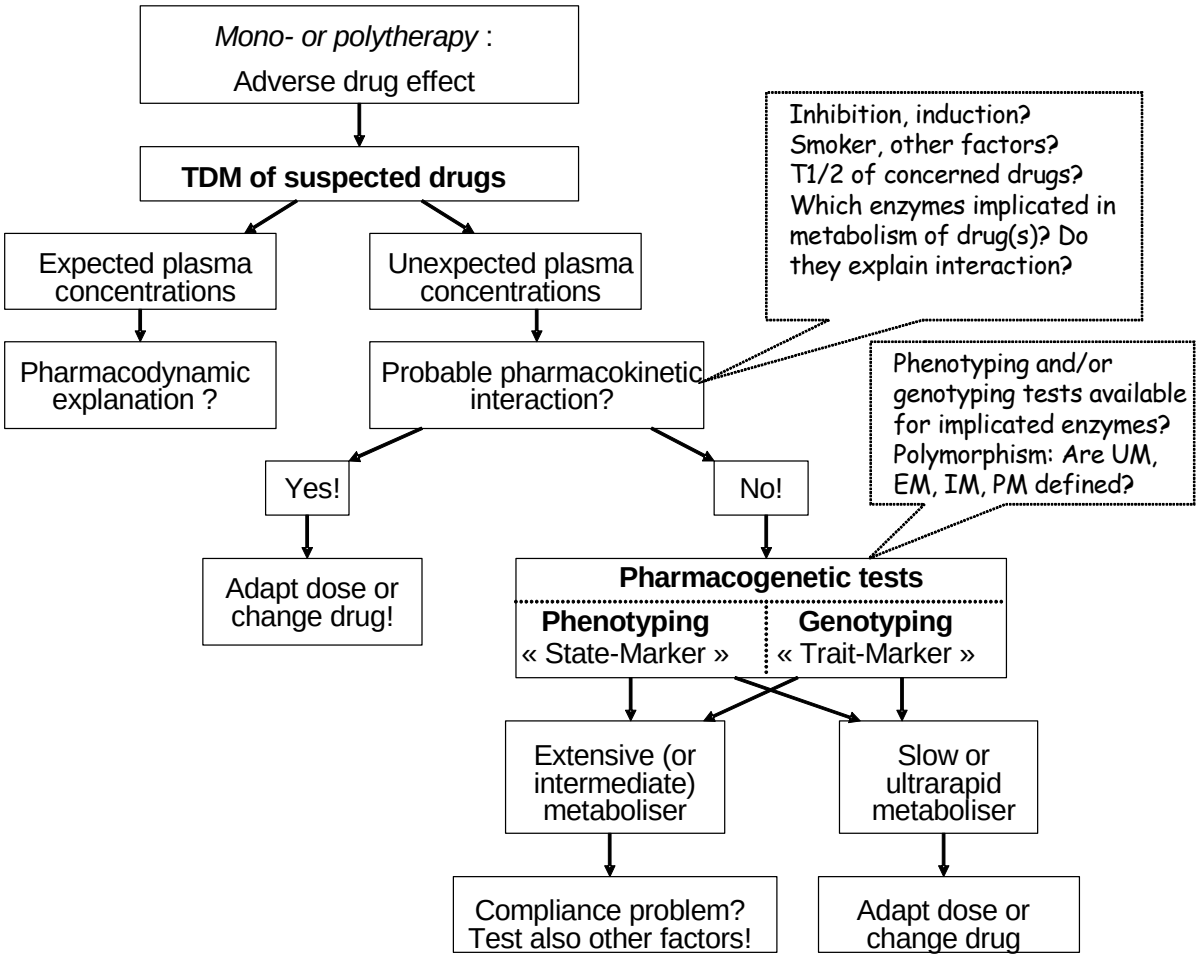
Although a case-control study seems an adequate study design for a heterogenic patient population, the nested case-control study was limited by the relative low number of cases and controls within one

in-patient hospital cohort. A case-control study in the entire AMSP cohort would generate far more cases but most of the clinics who participate in the AMSP project do this mainly for reasons of quality assurance and are not always interested in or able to perform research, many times also because there are no personnel resources for clinical research.

TDM plus

On the base of the experience gained in the AMSP+ study and the fact that we have only sparse data indicating that routine drug plasma concentration monitoring and/or pharmacogenetic testing (347) in psychiatry are justified to minimize the risk for ADR, we developed a test algorithm - which could be named "TDM plus" (TDM plus interaction checks plus pharmacogenetic testing) - on how to proceed in presence of an ADR (fig. 18) (64).

Figure 18: TDM plus algorithm



When a patient experiences an adverse drug effect, especially when serious or unexpected, drug plasma levels of suspected drugs are analysed. When the plasma levels are expected (dependent on the administered dose) a pharmacodynamic explanation is probable. If unexpectedly high or low a pharmacokinetic explanation should be considered.

Firstly the possibility of a pharmacokinetic drug interaction is examined. It is important to know all drugs taken, also oral contraception or over-the-counter preparations and herbal medication. Also prior medication, depending on its half-life, can be important. Furthermore lifestyle and diet should be taken into account: most importantly smoking, grapefruit, and consumption of alcohol or illegal drugs. All this information is checked in a drug interaction program reliable for its pharmacokinetic information (see chapter 5). Alternatively, the pharmacokinetic characteristics such as metabolism and transport pathways of the suspected drug or drug combination are looked up in the summary of product characteristics of each drug or in text books and the interaction potential is estimated.

If no pharmacokinetic drug interaction is found and the suspected drug(s) are metabolised by a polymorphic enzyme (e.g. CYP450) a pharmacogenetic test should be proposed. As explained in the introduction, genotyping is a trait marker but not available for all CYP450 enzymes. Phenotyping shows the activity of the tested enzyme, is a state marker and dependent on environment (here e.g. the drug taken). When genotyping is done, the information should be written in the patient's history and also given to the patient for all future treatment. Important tested alleles should be mentioned (fig.11). Pharmacogenetic tests exist also for other enzymes such UGT or for transporter proteins e.g. Pgp but the interpretation of their results is more difficult since less is known about substrate specificity and the clinical relevance of their different genotypes (64;348).

For some drugs routine TDM is recommended such as for lithium or clozapine (41), for other drugs with a narrow therapeutic index such as tricyclic antidepressants, TDM can prevent serious side effects (e.g. cardiac conduct disturbances, epileptic seizures, anticholinergic delirium), for all other drugs the proposed TDM plus algorithm seems, especially in the light of the health economical situation, more realistic.

Recommendations for further studies

A case-cohort study might help to overcome the problems we encountered to match controls to our cases, and inclusion of more cases will probably generate a clearer result concerning CYP2D6 genotype dependent (S)ADR risk. Limiting the heterogeneity of the cases in choosing either for a certain ADR group such as EPS or for a certain drug or drug group such as antipsychotics or antidepressants would be advisable as well. If one chooses a matched control design a more

homogenous patient group is advisable in order to reach the necessary number of matched case-control pairs to perform a more detailed risk analysis by logistic regression tests.

Written informed consent for drug plasma level monitoring, pharmacogenetic testing and use of these data for research purposes is ideally obtained of all patients of the clinic as soon as possible after admission. Blood samples for pharmacogenetic tests of all patients are taken; however, analyses are only done when needed. In this way costs are limited.

In conclusion, case-control or case-cohort studies seem appropriate to study drug safety risk factors such as pharmacokinetics' influencing genotypes or too high plasma concentrations of psychotropic medication. A recent state of the art paper on pharmacogenetic studies concluded that in rare and severe adverse drug reactions, case-control studies might be the sole feasible design (349).

7 mediQ.ch, a Web based Drug-Drug Interaction Database

Summary

The internet based drug interaction program mediQ.ch (www.mediQ.ch) meets the need of the clinicians for an easily accessible tool to rapidly check the risks of drug and drug combination therapies. Different levels of information depth allow rapid checks for significant interaction risks for uncomplicated treatments as well as more detailed information for complex cases. The information source for drug information and for the risk estimation is referenced and direct links to Pubmed allow access to the abstract of the source publications.

Risk estimation of drug combinations is mostly made for drug pairs, based on published data of clinical studies, case reports and drug characteristics. Pharmacokinetic and –dynamic as well as the side effect profile are taken into account.

Risk estimation is also provided for the influence of diet, lifestyle or pharmacogenetic factors. In the summary of the drug profile, mainly based on the summary of product characteristics, the clinician finds also information on the need of dose adaptation in case of renal or hepatic insufficiency, the potential for QT prolongation and for lowering the seizure threshold. In addition detailed and referenced information is given on the metabolic and transport pathways of the active substances described.

The main difference with other drug interaction programs is risk estimation for each drug pair in contrast to risk estimations based on class effects. This has the advantage to be more accurate and the disadvantage of generating a large amount of data which has to be, and is, regularly updated.

The program was originally set up by the author of this thesis to serve the clinicians of the psychiatric in-patient hospital Königsfelden but in the meantime it is implemented in over 100 hospitals in Switzerland, Germany and Austria and several hundred private practices.

Introduction

Unintended adverse drug effects are a more frequent cause of death than traffic accidents in developed countries(350). It is estimated that 200 to 700 cases of unintended adverse drug reactions occur in a hospital with 500 beds per year, and the extra costs per year reach an amount of 400 million EUR in Germany alone (351). In Switzerland, a study from 1999 estimated the costs of drug-related hospital admissions to 70-100 million Swiss francs (352).

The main reasons for serious adverse drug reactions are prescription errors, e.g. to incorrect dosage, double prescription, wrong medication and harmful drug-drug interactions (DDI) (353-356) which are either unknown or not considered. About 10% of all severe unintended adverse drug effects are due to adverse DDIs, in the elderly this increases to 15-20% (357;358). DDI are a major medical problem.

This becomes more important as polypharmacy becomes more common; patients are treated for several conditions in parallel and more therapies build on combination therapy (e.g. HIV, cancer, infectious diseases, cardiovascular, and psychiatric disorders). Polypharmacy is also driven by the increase of age of the population in the developed world (i.e. more patients with multiple conditions to be treated), and the increased number of available drugs. Therefore, at the same error rates, harmful DDI will increase if no tools are developed to support the prescription process in order to prevent harmful effects to patients.

Combination pharmacotherapy is most common today in psychiatry. According to different studies only 20% of the hospitalised patients get a psychotropic monotherapy (8;359)(data from the AMSP database), the mean being 3.5 and 5 for patients under 65 and over 65 years old, respectively (data from the AMSP database). Besides the aimed benefit there is also the risk of potentially harmful DDI. It is estimated that clinicians identify 20 to 40 % of potentially hazardous interactions (360;361). Taking into account that the Swiss market comprises about 6000 medicinal drugs with about 2000 active substances there are 2 million combination pairs (2 drugs) possible. Cautious calculations estimate the number of potentially harmful combinations to 40' – 70'000 (Chistoph Hiemke, personal communication) taken all medication together and about 7000 in psychiatry only. These calculations do not take additive risks of multiple drugs' combinations into account, nor interactions with food, lifestyle, genetic background and health status of the patient.

Pharmacogenetic factors further complicate the prescription process

Comparison of the list of drugs most commonly implicated in adverse drug reactions with the list of metabolizing enzymes with known polymorphisms shows that drugs commonly involved in adverse drug reactions were also those that were metabolized by enzymes with known polymorphisms (162). For certain new drugs pharmacogenetics is already part of standard practice for prescriptions (64;362;363). Thus, the clinician ideally knows the patient history, effects of food and life style, the exact medication with potential DDI, and in addition the potential effect of a certain pharmacogenetic status to determine whether a prescription is safe.

Information Technology (IT) support systems

To manage the overwhelming amount of data on potential drug effects, health professionals use more and more IT-based decision support systems when making prescriptions. Current databases which are

included in IT-based decision support systems list drug-drug interactions on one hand based on past observations of adverse effects in clinical practice, on the other hand, drugs are not treated individually but as members of an “effectors’ class”.

The volume and complexity of knowledge and information e.g. through extended drug innovations or front line research insights such as in the above mentioned pharmacogenetics require the use of IT-systems to support the prescription process. In general, medical practitioners lack behind other professions in implementing IT to support processes and share information. For example, only 14 % of all General Practitioners in Switzerland use electronic patient files (personal communication from e-mediat at e-health conference 2010). Estimations from the US say that that the use of IT could prevent 2 million adverse drug interactions and subsequent 190’000 hospitalisations a year (364).

The US government therefore plans regulations to make electronic prescriptions (as opposed to paper prescriptions) mandatory by 2011. Prescription devices or systems will have to include an automatic check for interaction risk assessments. In Europe, similar regulation is likely to follow. Several initiatives are underway in Switzerland to overcome this systemic weakness, with the electronic patient files (e.g. a “patient smart card”) as one of the steps.

Concept of the interaction program mediQ.ch

The program has been designed and built to meet the need of the clinicians for an easily accessible source of reliable information where they can rapidly check the risks of drug and drug combination therapies. Different levels of information depth does allow the rapid check for significant interaction risks for uncomplicated treatments as well more detailed information for complex cases. The user of the program should always be able to know on which source the information of the risk estimation is based on, so information is referenced.

The program does also inform (at least partly) on the interactions of patient factors such as pharmacogenetic status, renal and hepatic insufficiency, and the risk for long Qt syndrome or lowered convulsion threshold with a certain drug or drug combination treatment.

The mediQ.ch database is built “bottom-up” on pharmacological data (fig. 19), which allows integrating pharmacogenetic and other new information as it is established. Thus, the data base can be extended easily by the “genetics” dimension or e.g. by the influence of co-morbidities, which is not possible – or very laborious – in other data bases. In addition, new drugs can be easily assessed for potential interactions when their metabolism and mode of action are known.

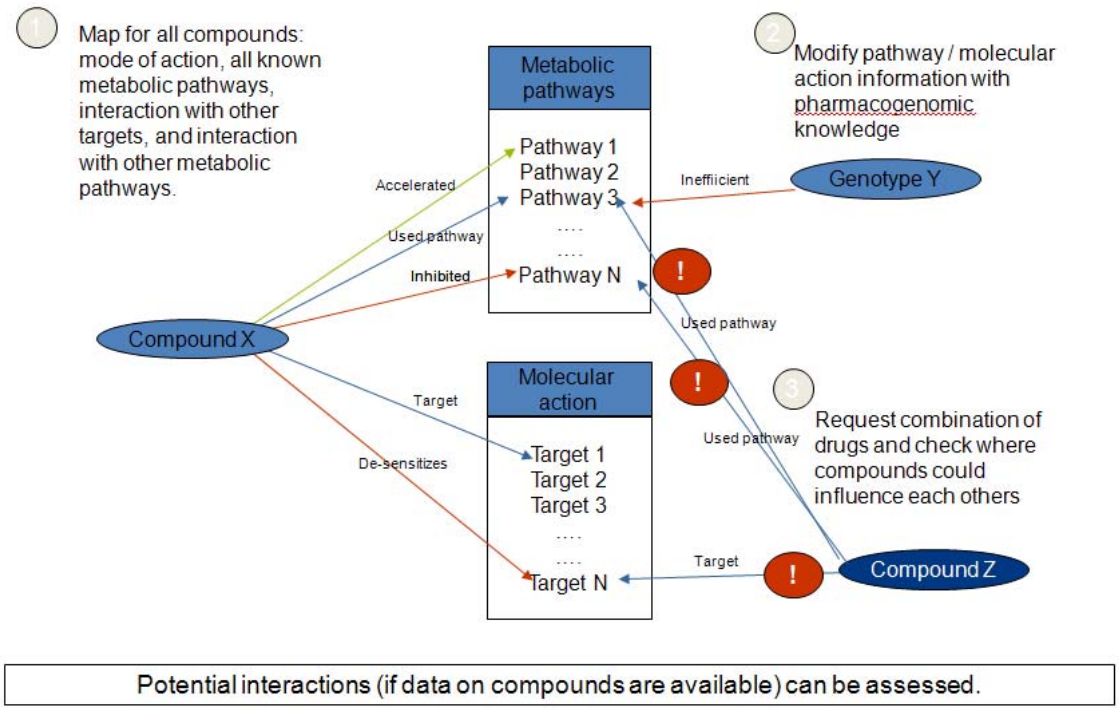
For this deductive and mechanistic approach, mediQ.ch has filled an outstanding database on the pharmacological profile of pharmaceutical compounds and pharmacogenetic data, including detailed

information for about 20’000 interactions, 5’000 drugs, 2’000 substances, 400 indication classes, 150 metabolic/transport pathways and 25 genotypes, including links to original scientific literature.

The mediQ.ch source data is retrieved from the summary of medicinal product characteristics for healthcare professionals and published medical literature, based wherever possible on clinical data. The corresponding literature references are indicated. In order to keep up with the new research results, mediQ.ch’s data-base is continuously online updated and extended. Furthermore, several research collaborations help mediQ.ch to stay abreast of the scientific development (Unité de Biochimie et Psychopharmacologie Clinique, Centre des Neurosciences Psychiatriques of the University of Lausanne, IKC of the University of Zürich, and Psychiatric University Institute in Munich amongst others).

Thus, the mediQ.ch system is an easy to use tool to access the fundamental data relevant to assess drug interaction risks with the ability to integrate new biomedical insights in a dynamic and intuitive way.

Figure 19: Estimation of interactions potential on pharmacological bases (Number of potential interaction pairs of “n” drugs: n(n-1)/2: At 2’000 known compounds this yields 2 millions interactions, not taking into account genotypes.)*



Information Sources

Besides the prescriber's information or Summary of product characteristics (SPC) the following sources are used:

1. Drug interaction/combination studies in patients: these studies can give a proper insight in the expected interactions in the studied patient population. Importantly, one has to consider that drugs are sometimes used in different indications, with a different patient population and therefore different risks. If these studies show no clinically relevant interaction, vulnerable patients could still be at risk.
2. Drug interaction studies in healthy volunteers: These studies are mostly performed to study pharmacokinetic interactions, pharmacodynamic effects are sometimes neglected. Healthy volunteers are in general less vulnerable to adverse drug reactions than patients.
3. Case reports: case reports are a valuable source of clinical information on the potential risk for rare adverse events of a drug combination.
4. Pharmacological information is retrieved from the prescriber's information and pharmacological studies, mainly found through literature search in Pubmed or in other drug interaction programs such as Micromedex (Thomson Micromedex, Thomson Reuters 1974-2010**)** or Genelex (www.genemedrx.com).
   4.1. Pharmacokinetic information (absorption, distribution, metabolism, excretion)
      4.1.1. In vivo: it is important to extensively search for in vivo data, since it is difficult from in vitro data to predict the in vivo activities.
      4.1.2. In vitro: where no in vivo data are available in vitro information is taken. However, the interpretation is done more cautiously than with in vivo data.
   4.2. Pharmacodynamic information (e.g. receptor affinity, agonistic/antagonistic action): pharmacodynamic information is sometimes difficult to find and interpret. In 2010 this information is still mostly missing in mediQ.ch.
5. Side effect profile: the risk for certain drug reactions can substantially increase (accumulated risk) when more than one drug bears this risk (e.g. prolongation of QT-interval and risk of torsade de pointes; liver enzyme disturbances; electrolyte disturbances; neurotoxicity etceteras.)

Literature searches are mostly done in Pubmed, supported by e-alerts from scientific journals and alerts from drug safety authorities such as FDA, EMEA and Swissmedic

Concise risk estimation

Concise risk estimation comments are written by experienced health professionals (pharmacists, physicians, pharmacologists…) and corrected by at least one peer (minimum 4 eyes). All information is updated as new data become available and periodically, normally all 2 years.

In cases where no clinical information is found, mediQ's risk estimation is compared to the information in other drug interaction programs, e.g. Micromedex, Genelex, Pharmavista (www.pharmavista.net), PSIAC (www.psiac.de).

Structure of the mediQ.ch interaction program

The program is web-based in order to be easily accessible, always updated, dynamic with links to other information sources and interactive with online advice on specific questions

Core of the program is the interaction-check where two or more substances can be combined, also with information on genetic polymorphisms. The answers will be presented in different ways and amount of details (see examples with screen shots below).

In addition each substance is described with a summary of the prescriber's information, important safety information (dose decrease in renal or hepatic insufficiency, potential for QT-prolongation, lowering of seizure threshold), pregnancy category , and detailed information on metabolism and transport of the drug,

There is also information in which galenic formulation under which trade name a substance is available.

All information is accessible via the name of the active substance or the trade name of the medicinal drug.

As a supplement a glossary with useful definitions and links to other information sources in the field of drug interactions are given.

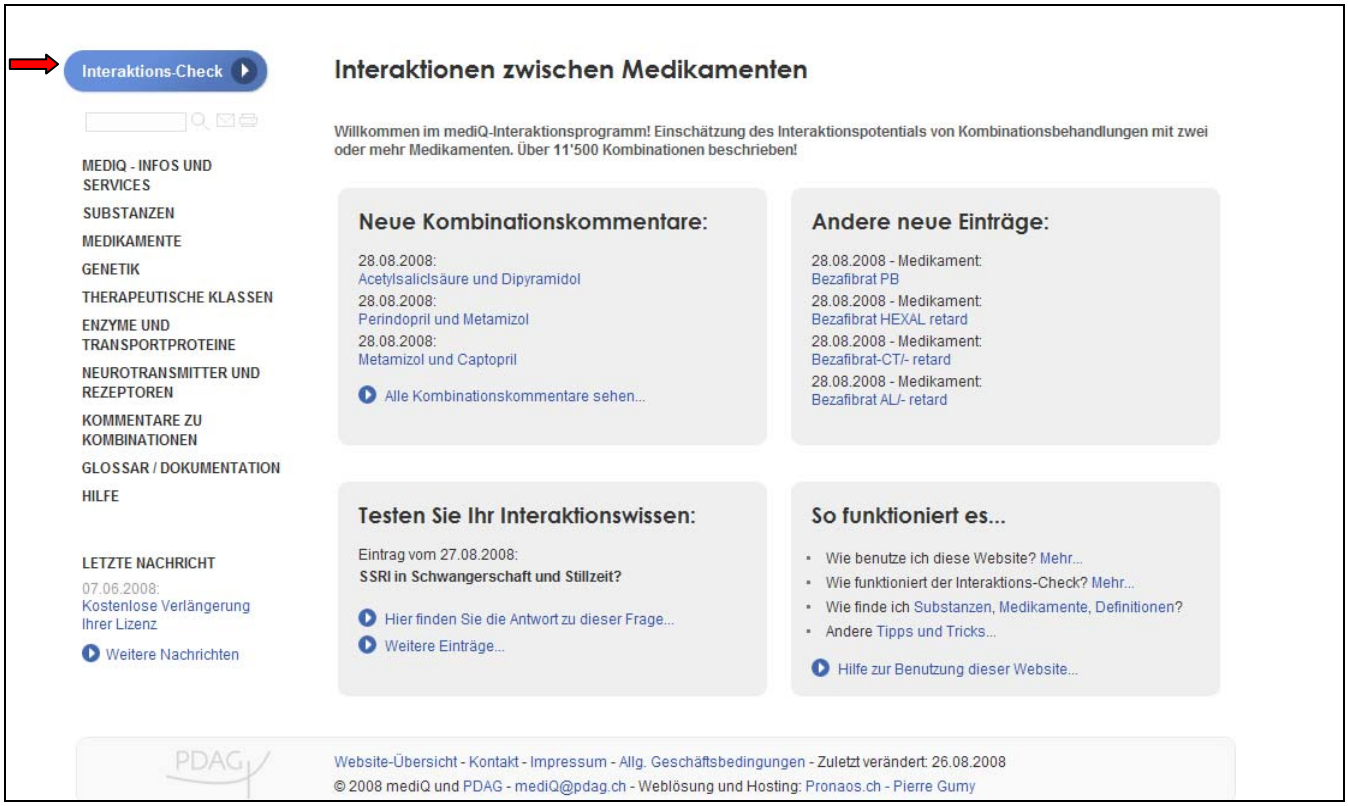
The user guide explaining all the features is accessible on www.mediQ.ch under "So funktioniert es.... Screenshots und Präsentationen".

Examples

Example 1

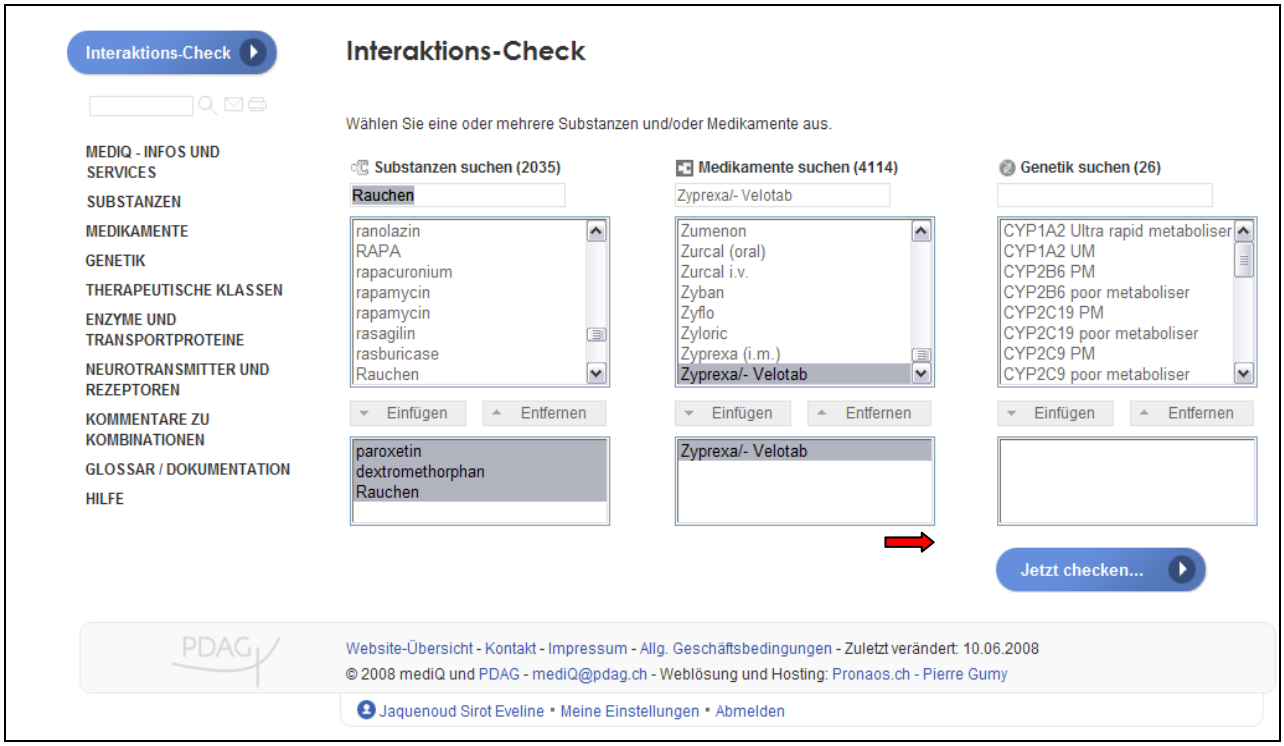
A 40 year old male patient suffering from schizoaffective disorder, under olanzapine treatment needs an antidepressant, paroxetine being the first choice. The same patient suffers from a dry cough and he asks for a cough syrup containing dextromethorphan. He also mentions that he considers stop smoking. No other health problems are known.

Figure 20: First page after log in of the drug interaction program mediQ



The entry page (fig. 20) displays on the left side different search categories, most important in blue the page which leads to the interaction check of drug combinations. We choose “Interaktions-Check”.

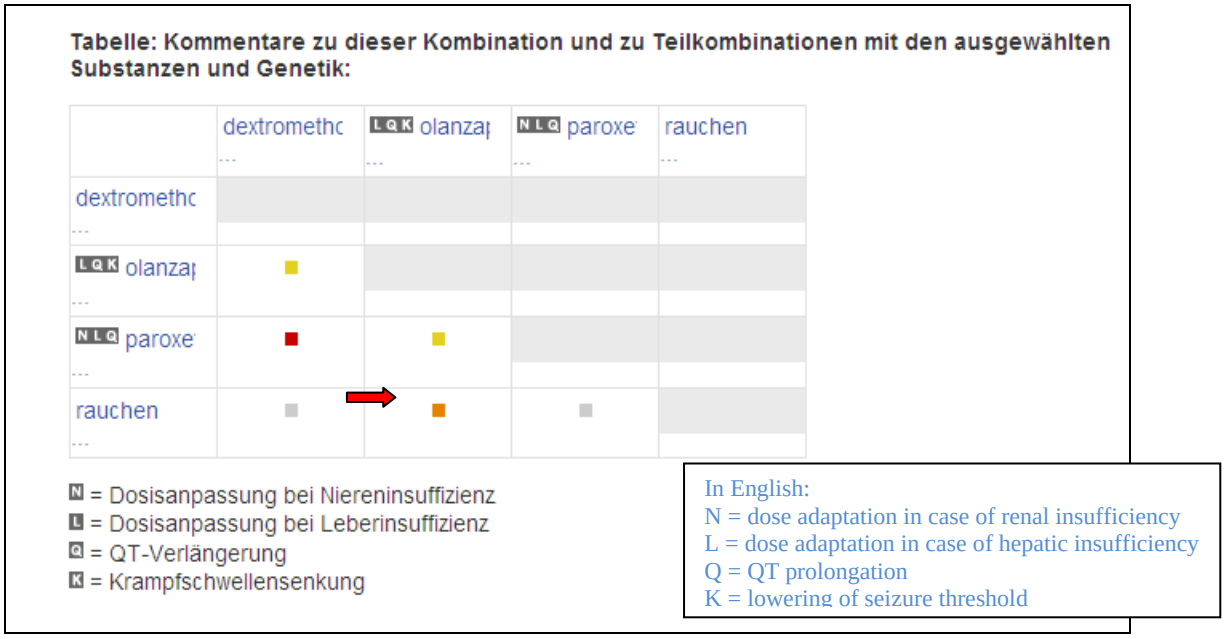
Figure 21: Drug entry page for the interaction check in mediQ.ch



On the page “Interaktions-Check” (fig. 21) active substances (“Substanzen”) and/or drugs with their trade name (“Medikamente”) and/or a pharmacogenetic status (“Genetik”) can be combined. The 3 drugs of our example and smoking (“Rauchen”) are chosen for checking the interactions under „Jetzt checken“.

First an overview (fig. 22) of the clinical relevance of a potential interaction per drug pair is found. Red, meaning “highly relevant interaction, often also contra-indicated”, orange: “clinically meaningful”, yellow: “might be relevant in special clinical situations or vulnerable patients”, gray: no clinically relevant interaction expected. Besides that the user can see if there are relevant issues around liver (L) or renal (N) insufficiency, potential for QT-prolongation (Q) or lowering of the seizure threshold (K).

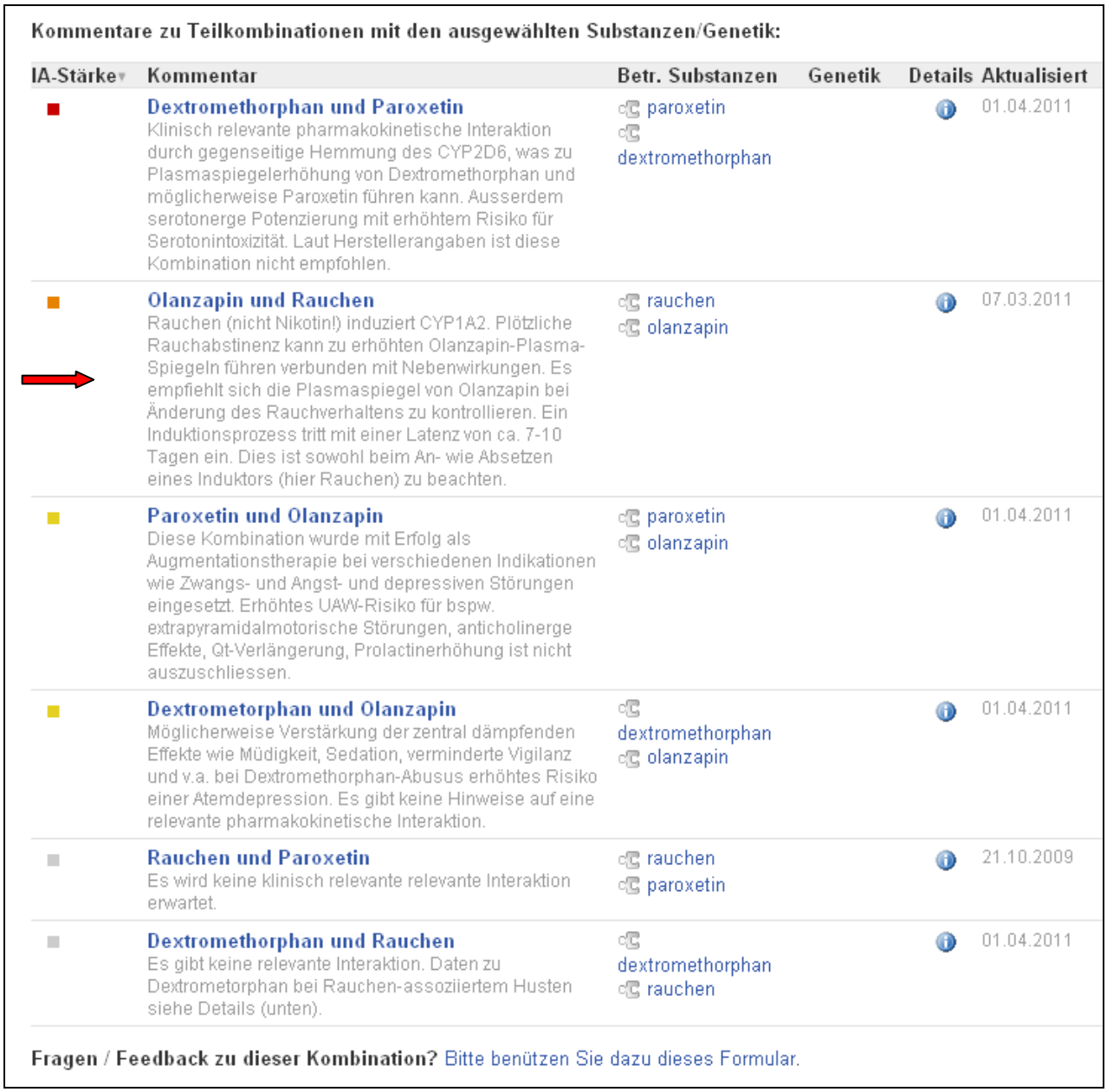
Figure 22: graphic overview of the relevance of potential interactions between the chosen substances



In our example we find a highly relevant interaction between dextromethorphan and paroxetine, a clinically relevant interaction between smoking and olanzapine, two weak interactions between olanzapine and dextromethorphan and paroxetine respectively. We further see that for paroxetine and olanzapine we need to adapt the dose in cases of renal or hepatic insufficiency; further that both drugs have a certain potential for QT prolongation and that olanzapine lowers the seizure threshold.

We now have the choice to click on one of combinations either in the first, or as seen in the next slide (fig. 23), second overview. We want to know what effect smoking has on olanzapine and click on the second combination comment.

Figure 23: Second overview with summary of the potential interaction per drug pair is listed under the first overview.



Kommentare zu Teilkombinationen mit den ausgewählten Substanzen/Genetik:

IA-Stärke	Kommentar	Betr. Substanzen	Genetik	Details	Aktualisiert
■	**Dextromethorphan und Paroxetin** Klinisch relevante pharmakokinetische Interaktion durch gegenseitige Hemmung des CYP2D6, was zu Plasmaspiegelerhöhung von Dextromethorphan und möglicherweise Paroxetin führen kann. Ausserdem serotonerge Potenzierung mit erhöhtem Risiko für Serotonintoxizität. Laut Herstellerangaben ist diese Kombination nicht empfohlen.	paroxetin dextromethorphan			01.04.2011
■	**Olanzapin und Rauchen** Rauchen (nicht Nikotin!) induziert CYP1A2. Plötzliche Rauchabstinenz kann zu erhöhten Olanzapin-Plasma-Spiegeln führen verbunden mit Nebenwirkungen. Es empfiehlt sich die Plasmaspiegel von Olanzapin bei Änderung des Rauchverhaltens zu kontrollieren. Ein Induktionsprozess tritt mit einer Latenz von ca. 7-10 Tagen ein. Dies ist sowohl beim An- wie Absetzen eines Induktors (hier Rauchen) zu beachten.	rauchen olanzapin			07.03.2011
■	**Paroxetin und Olanzapin** Diese Kombination wurde mit Erfolg als Augmentationstherapie bei verschiedenen Indikationen wie Zwangs- und Angst- und depressiven Störungen eingesetzt. Erhöhtes UAW-Risiko für bspw. extrapyramidalmotorische Störungen, anticholinerge Effekte, Qt-Verlängerung, Prolactinerhöhung ist nicht auszuschliessen.	paroxetin olanzapin			01.04.2011
■	**Dextrometorphan und Olanzapin** Möglicherweise Verstärkung der zentral dämpfenden Effekte wie Müdigkeit, Sedation, verminderte Vigilanz und v.a. bei Dextromethorphan-Abusus erhöhtes Risiko einer Atemdepression. Es gibt keine Hinweise auf eine relevante pharmakokinetische Interaktion.	dextromethorphan olanzapin			01.04.2011
■	**Rauchen und Paroxetin** Es wird keine klinisch relevante relevante Interaktion erwartet.	rauchen paroxetin			21.10.2009
■	**Dextromethorphan und Rauchen** Es gibt keine relevante Interaktion. Daten zu Dextrometorphan bei Rauchen-assoziiertem Husten siehe Details (unten).	dextromethorphan rauchen			01.04.2011

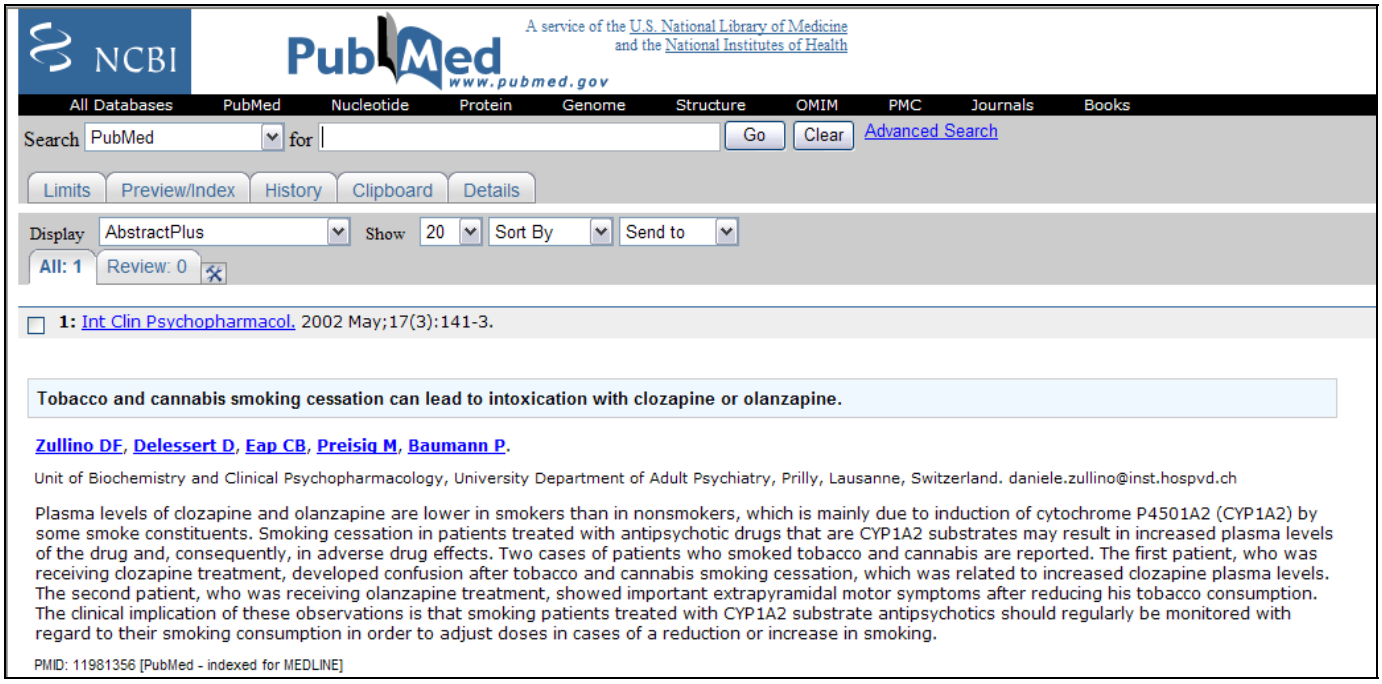
Fragen / Feedback zu dieser Kombination? Bitte benützen Sie dazu dieses Formular.

We click on the second combination “olanzapine and smoking” in order to obtain the detailed description of this combination comment (fig. 24).

Figure 24: Detailed combination comment on the influence of smoking on olanzapine treatment with information source references

The comment explains that smoking (not nicotine) induces CYP1A2 and that sudden smoking cessation without dose adaptation can lead to high olanzapine plasma concentration and high risk for adverse drug reactions. It also mentions that the induction effect comes with latency. Clicking on a reference (e.g. Zullino DF et al 2002) brings us to the abstract of the source information (fig. 25).

Figure 25: abstract of source reference from the open access literature service "Pubmed"



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Display AbstractPlus Show 20 Sort By Send to

All: 1 Review: 0

1: Int Clin Psychopharmacol. 2002 May;17(3):141-3.

Tobacco and cannabis smoking cessation can lead to intoxication with clozapine or olanzapine.

Zullino DF, Delessert D, Eap CB, Preisig M, Baumann P.

Unit of Biochemistry and Clinical Psychopharmacology, University Department of Adult Psychiatry, Prilly, Lausanne, Switzerland. daniele.zullino@inst.hospvd.ch

Plasma levels of clozapine and olanzapine are lower in smokers than in nonsmokers, which is mainly due to induction of cytochrome P4501A2 (CYP1A2) by some smoke constituents. Smoking cessation in patients treated with antipsychotic drugs that are CYP1A2 substrates may result in increased plasma levels of the drug and, consequently, in adverse drug effects. Two cases of patients who smoked tobacco and cannabis are reported. The first patient, who was receiving clozapine treatment, developed confusion after tobacco and cannabis smoking cessation, which was related to increased clozapine plasma levels. The second patient, who was receiving olanzapine treatment, showed important extrapyramidal motor symptoms after reducing his tobacco consumption. The clinical implication of these observations is that smoking patients treated with CYP1A2 substrate antipsychotics should regularly be monitored with regard to their smoking consumption in order to adjust doses in cases of a reduction or increase in smoking.

PMID: 11981356 [PubMed - indexed for MEDLINE]

Under the second overview we find a rough overview of CYP450 interactions (fig.26). The variation in bioavailability is not yet taken into account or some other factors such as where different CYP450 isoforms are active (e.g. intestinal vs. hepatic...).

Figure 26: Rough overview of CYP450 interactions

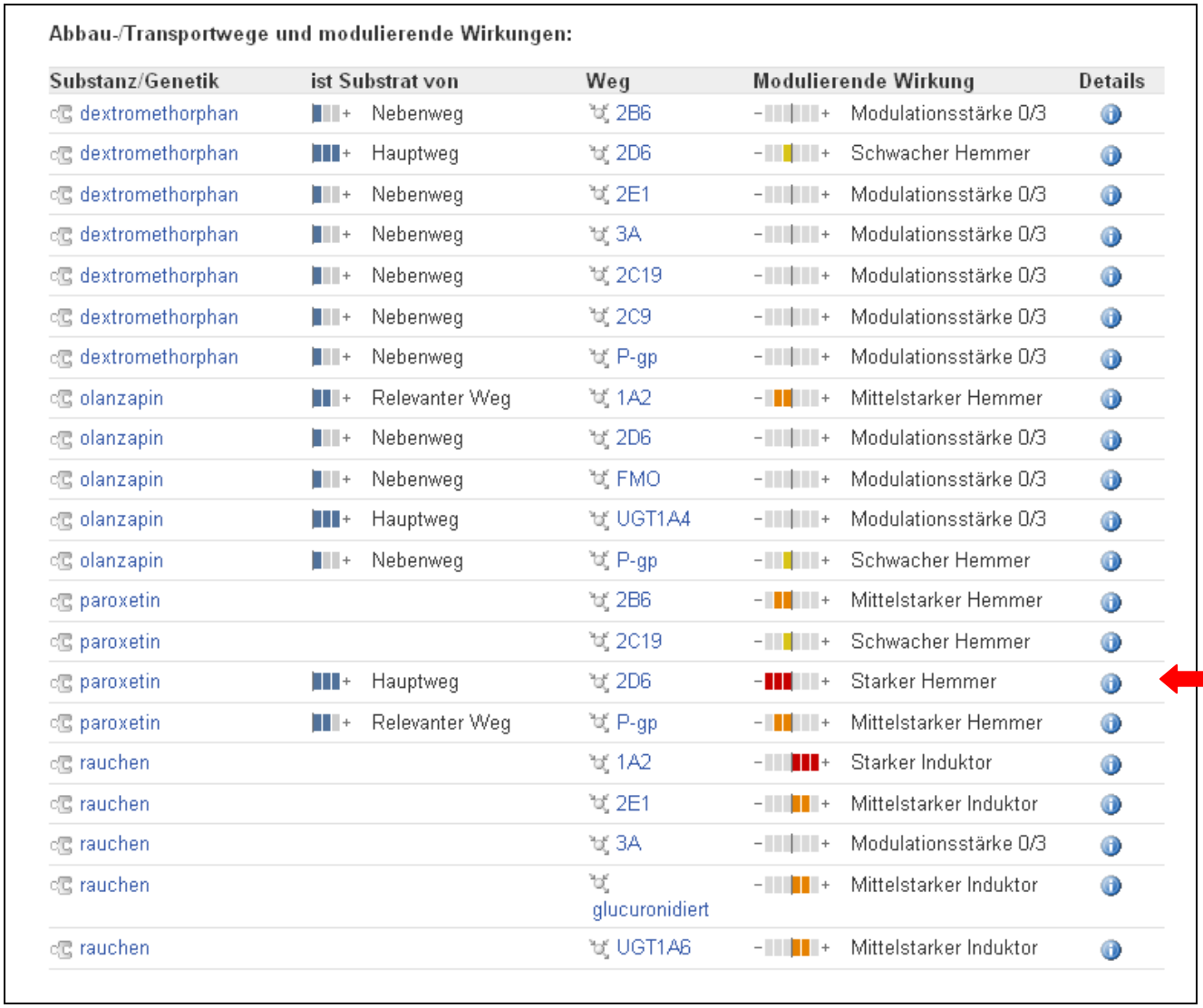
CYP450 Interaktionen:

Grobeinschätzung, je nach Bioverfügbarkeit und alternativer Abbauwege können Abweichungen entstehen.

Betroffen	Änderung der Blutkonzentration	Verursacher	Weg
dextromethorphan	Minime Hemmung bei Nebenweg: keine relevante Interaktion	paroxetin	2C19
dextromethorphan	Relevante Induktion bei Nebenweg	rauchen	2E1
dextromethorphan	Starke Hemmung: Plasmaspiegelerhöhung	paroxetin	2D6
dextromethorphan	Relevante Hemmung bei Nebenweg	paroxetin	2B6
olanzapin	Starke Hemmung bei Nebenweg: Plasmaspiegelerhöhung	paroxetin	2D6
olanzapin	Minime Hemmung bei Nebenweg: keine relevante Interaktion	dextromethorphan	2D6
olanzapin	Starke Induktion, mehrere Wege: Plasmaspiegelsenkung	rauchen	1A2
paroxetin	Minime Hemmung, vermutlich klinisch nicht relevant	dextromethorphan	2D6

Below an overview of metabolic and transport pathways with importance of substrate affinity and strength of modulating activity is found (fig. 27).

Figure 27: Overview of metabolic and transport pathways with substrate affinity and modulating effects

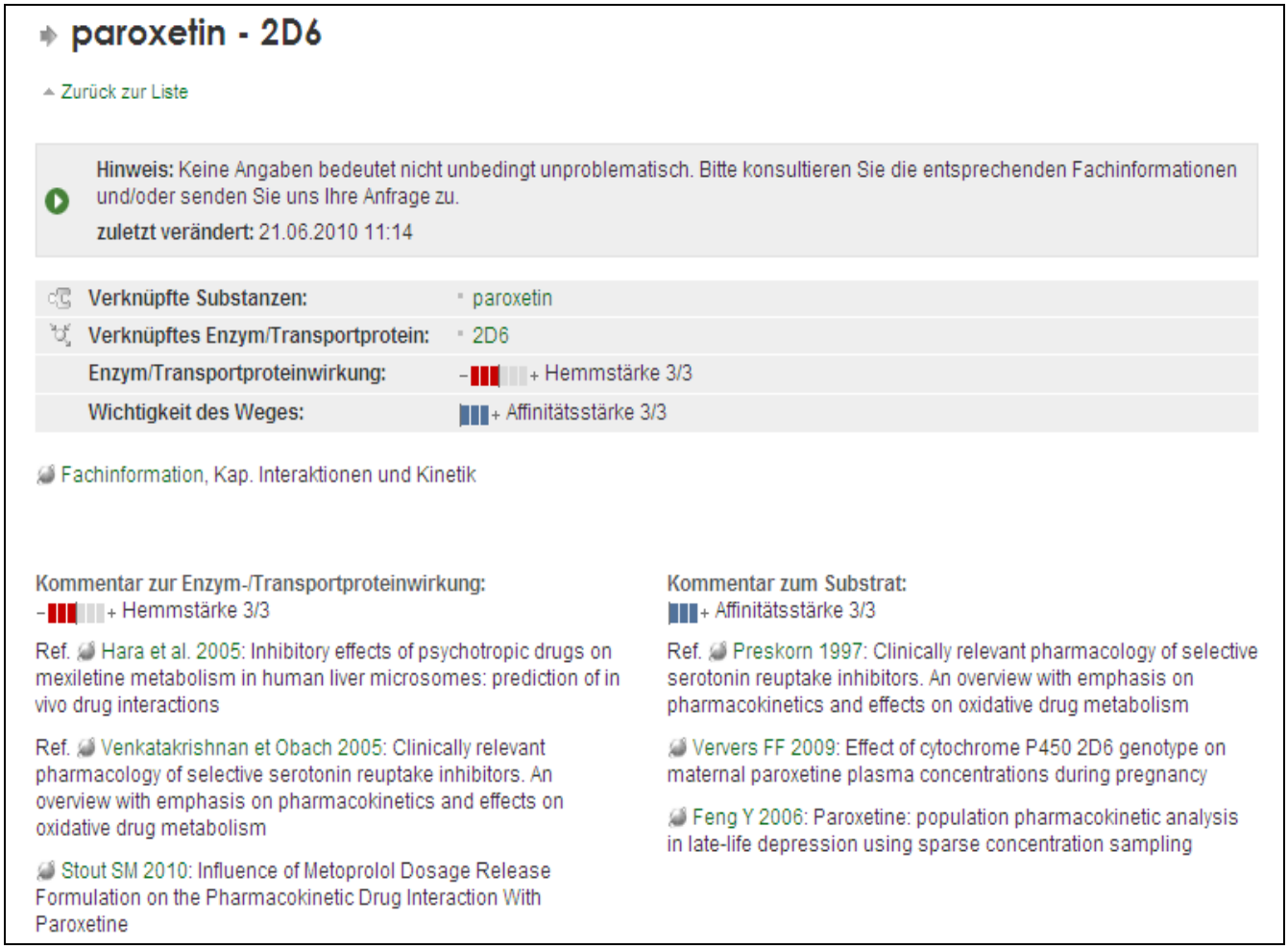


Abbau-/Transportwege und modulierende Wirkungen:

Substanz/Genetik	ist Substrat von	Weg	Modulierende Wirkung	Details
dextromethorphan	Nebenweg	2B6	Modulationsstärke 0/3	
dextromethorphan	Hauptweg	2D6	Schwacher Hemmer	
dextromethorphan	Nebenweg	2E1	Modulationsstärke 0/3	
dextromethorphan	Nebenweg	3A	Modulationsstärke 0/3	
dextromethorphan	Nebenweg	2C19	Modulationsstärke 0/3	
dextromethorphan	Nebenweg	2C9	Modulationsstärke 0/3	
dextromethorphan	Nebenweg	P-gp	Modulationsstärke 0/3	
olanzapin	Relevanter Weg	1A2	Mittelstarker Hemmer	
olanzapin	Nebenweg	2D6	Modulationsstärke 0/3	
olanzapin	Nebenweg	FMO	Modulationsstärke 0/3	
olanzapin	Hauptweg	UGT1A4	Modulationsstärke 0/3	
olanzapin	Nebenweg	P-gp	Schwacher Hemmer	
paroxetin		2B6	Mittelstarker Hemmer	
paroxetin		2C19	Schwacher Hemmer	
paroxetin	Hauptweg	2D6	Starker Hemmer	
paroxetin	Relevanter Weg	P-gp	Mittelstarker Hemmer	
rauchen		1A2	Starker Induktor	
rauchen		2E1	Mittelstarker Induktor	
rauchen		3A	Modulationsstärke 0/3	
rauchen		glucuronidiert	Mittelstarker Induktor	
rauchen		UGT1A6	Mittelstarker Induktor	

In case we want to know on what information sources mediQ relies for e.g. CYP2D6 and paroxetine we will find that in clicking on the right information button under details (fig. 28).

Figure 28: Source references, all with direct link (click on the author name in green) to their abstract in the open access literature service "Pubmed" or link to the journal, especially in case where free full text is available.

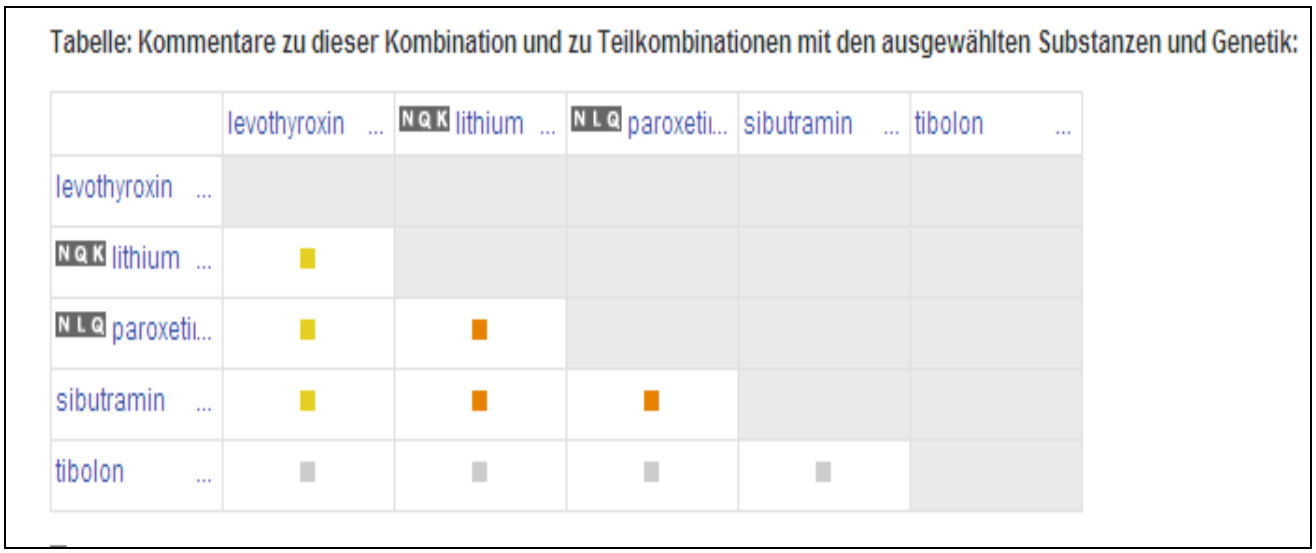


Example 2

A 54 year old female bipolar patient consults because she fears experiencing another manic episode. She presents with profuse and for her untypical sweatening; she feels restless, suffers from handicapping tremor (cannot handle the computer mouse, cannot hold a cup without spilling...). A month ago she added some new drugs to her existent medication after consulting a gynaecologist for menopausal complaints and weight gain. Her medication presented as follows: since several years: paroxetine 20mg/d, lithium 660mg/d, and levothyroxine 0.05mg/d; since 4 weeks tibolone 2.5mg/d against menopausal symptoms and sibutramine 10mg/d to reduce her weight gain.

The result of the interaction check presents as follows with the first graphic overview (fig. 29), below the second overview with the summaries of the most relevant information for each combination pair (fig. 30) and finally a detailed comment for the combination of lithium with paroxetine (fig. 31).

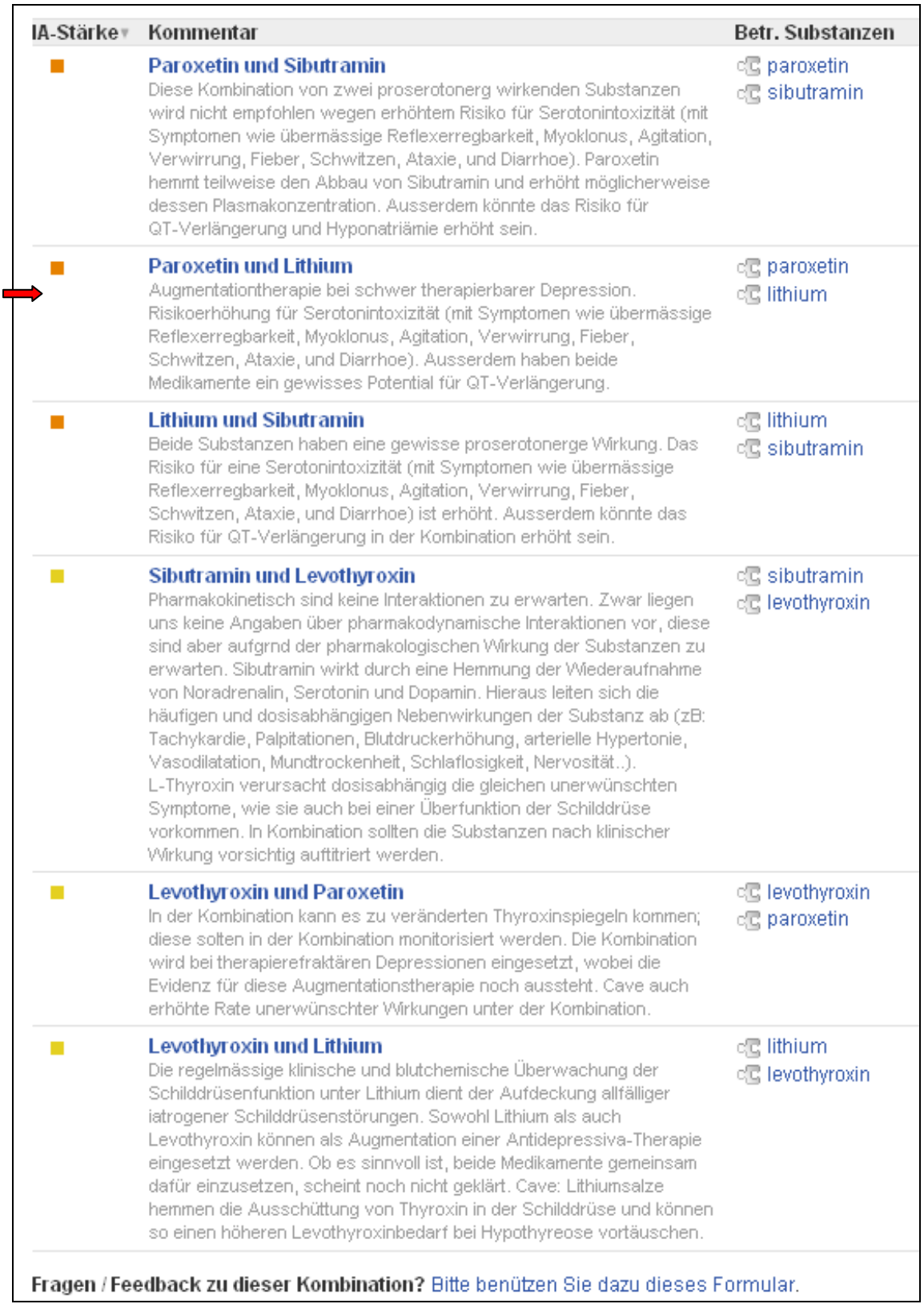
Figure 29: Graphic overview of the interaction risk of the combination pairs in the combination treatment of paroxetine, lithium, levothyroxine and sibutramine



The 3 orange flagged combination pairs show a serotonin agonistic potentiation of the sibutramin, paroxetine and lithium and warn of the risk of serotonin toxicity. mediQ.ch only displays comments on drug pairs; therefore clinician should read all the comments in order to get the full picture.

The summaries of the next overview (fig. 30) mention the most important symptoms of a serotonergic overstimulation such as hyperreflexia, myoclonus, agitation, confusional state, hyperthermia, sweatening, ataxia and diarrhoe, it shows also an increased risk for QT prolongation and hyponatriemia.

Figure 30: Summary overview for the drug pairs of the combination treatment with paroxetine, lithium, levothyroxine and sibutramine.



IA-Stärke	Kommentar	Betr. Substanzen
■	**Paroxetin und Sibutramin** Diese Kombination von zwei proserotonerg wirkenden Substanzen wird nicht empfohlen wegen erhöhtem Risiko für Serotonintoxizität (mit Symptomen wie übermässige Reflexerregbarkeit, Myoklonus, Agitation, Verwirrung, Fieber, Schwitzen, Ataxie, und Diarrhoe). Paroxetin hemmt teilweise den Abbau von Sibutramin und erhöht möglicherweise dessen Plasmakonzentration. Ausserdem könnte das Risiko für QT-Verlängerung und Hyponatriämie erhöht sein.	paroxetin sibutramin
■	**Paroxetin und Lithium** Augmentationtherapie bei schwer therapierbarer Depression. Risikoerhöhung für Serotonintoxizität (mit Symptomen wie übermässige Reflexerregbarkeit, Myoklonus, Agitation, Verwirrung, Fieber, Schwitzen, Ataxie, und Diarrhoe). Ausserdem haben beide Medikamente ein gewisses Potential für QT-Verlängerung.	paroxetin lithium
■	**Lithium und Sibutramin** Beide Substanzen haben eine gewisse proserotonerge Wirkung. Das Risiko für eine Serotonintoxizität (mit Symptomen wie übermässige Reflexerregbarkeit, Myoklonus, Agitation, Verwirrung, Fieber, Schwitzen, Ataxie, und Diarrhoe) ist erhöht. Ausserdem könnte das Risiko für QT-Verlängerung in der Kombination erhöht sein.	lithium sibutramin
■	**Sibutramin und Levothyroxin** Pharmakokinetisch sind keine Interaktionen zu erwarten. Zwar liegen uns keine Angaben über pharmakodynamische Interaktionen vor, diese sind aber aufgrnd der pharmakologischen Wirkung der Substanzen zu erwarten. Sibutramin wirkt durch eine Hemmung der Wiederaufnahme von Noradrenalin, Serotonin und Dopamin. Hieraus leiten sich die häufigen und dosisabhängigen Nebenwirkungen der Substanz ab (zB: Tachykardie, Palpitationen, Blutdruckerhöhung, arterielle Hypertonie, Vasodilatation, Mundtrockenheit, Schlaflosigkeit, Nervosität..). L-Thyroxin verursacht dosisabhängig die gleichen unerwünschten Symptome, wie sie auch bei einer Überfunktion der Schilddrüse vorkommen. In Kombination sollten die Substanzen nach klinischer Wirkung vorsichtig auftitriert werden.	sibutramin levothyroxin
■	**Levothyroxin und Paroxetin** In der Kombination kann es zu veränderten Thyroxinspiegeln kommen; diese solten in der Kombination monitorisiert werden. Die Kombination wird bei therapierefraktären Depressionen eingesetzt, wobei die Evidenz für diese Augmentationstherapie noch aussteht. Cave auch erhöhte Rate unerwünschter Wirkungen unter der Kombination.	levothyroxin paroxetin
■	**Levothyroxin und Lithium** Die regelmässige klinische und blutchemische Überwachung der Schilddrüsenfunktion unter Lithium dient der Aufdeckung allfälliger iatrogener Schilddrüsenstörungen. Sowohl Lithium als auch Levothyroxin können als Augmentation einer Antidepressiva-Therapie eingesetzt werden. Ob es sinnvoll ist, beide Medikamente gemeinsam dafür einzusetzen, scheint noch nicht geklärt. Cave: Lithiumsalze hemmen die Ausschüttung von Thyroxin in der Schilddrüse und können so einen höheren Levothyroxinbedarf bei Hypothyreose vortäuschen.	lithium levothyroxin

Fragen / Feedback zu dieser Kombination? Bitte benützen Sie dazu dieses Formular.

In figure 31 the details for the combination pair lithium and paroxetine is displayed. It mentions that this combination is used as augmentation therapy in patients with a difficult to treat depression but it bears a certain increased risk for serotonin toxicity and QT prolongation.

Figure 31: Detailed comment on the combination of paroxetine with lithium

For further information on serotonin toxicity a link will bring you to the glossary, as shown in figure 32.

Figure 32: Glossary text on serotonin toxicity

Serotonin Syndrom (Serotonin Toxizität)

Zurück zur Liste

Zentrale Überstimulierung des serotonergen Systems, klinisch gekennzeichnet durch Trias: 1. Neuromuskuläre Übererregbarkeit (Hyperreflexie, Klonus, Tremor); 2. Psychische Veränderung (Agitation); 3. Autonome Symptome (Fieber, Schwitzen)

Serotonin Toxizität: aktuelle Übersicht (Volltext) bei Stephan 2008. Ausführliche Informationen auf der Webseite von P.K. Gillman, dort kann kostenlos auch eine 100-seitige Dokumentation angefordert werden.

Entwicklung von diagnostischen Kriterien: Sternbach 1991: Review von 38 publizierten Fällen, davon 32 betreffend die Kombination von Fluoxetin, Clomipramin oder L-Tryptophan/(+teilweise Lithium) mit einem irreversiblen, nicht selektiven Monoaminooxidase Hemmer. Weitere 5 Fälle betreffend Fluoxetin + L-Tryprophan. Erster Vorschlag für diagnostische Kriterien:

Sternbach-Kriterien:

A: Koinzidenz der Symptomatik mit Therapiebeginn oder Dosissteigerung einer bekanntermassen serotonergen Substanz; mind. 3 der folgenden Symptome vorhanden: 1. Psychische Veränderung (Verwirrung, Hypomanie), 2. Agitation, 3. Tremor, 4. Hyperreflexie, 5. Myoklonus, 6. Ataxie, 7. Hyperhidrosis, 8. Ataxie, 9. Fieber, 10. Schüttelfrost

B: Andere Ursache ausgeschlossen (infektiös, metabolisch, Substanzmissbrauch- oder -Entzug)

C: Keine Zugabe oder Dosissteigerung eines Neuroleptikums vor Beginn der Symptomatik

Die Sternbach-Kriterieen sind unspezifisch und haben einen "entweder-oder"-Charakter. Das moderne Verständnis geht von einer kontinuierlich sich steigernden Symptomatik aus, abhängig von der zentralen, intrasynaptischen Serotonin-Konzentration (vgl. dazu Boyer 2005). Dieses "spectrum concept" wird mit der von Hegerl 1998 vorgeschlagenen "serotonin syndrome scale", erarbeitet an ausschliesslich mit Paroxetin behandelten Patienten, besser erfasst. Die Items sind praktisch identisch mit denjenigen von Sternbach.

Kriterien nach Hegerl:

9 Kriterien, bewertet von 0-3 Punkten, Serotonin Syndrom ab > 6 Punkten: 1. Agitation, 2. Orientierungsstörung, 3. Myoclonus, 4. Hyperreflexie, 5. Tremor, 6. Schwindel, 7. Fieber, 8. Schwitzen, 9. Diarrhoe

Die systematische Analyse von beinahe 500 Intoxikationsfällen mit (ausschliesslich) SSRI führte zu nochmals verbesserten diagnostischer Kriterien, welche sich gegenüber denjenigen von Sternbach durch eine höhere Sensitivität und Spezifität auszeichnen und in der Anwendung einfacher sind (Dunkley 2003, Volltext). Ein wichtiges klinisches Symptom ist dabei der Klonus. Das zentrale, bereits von Sternbach formulierte Kriterium der ST bleibt aber die notwendige Präsenz einer serotonergen Substanz (allein oder in Kombination).

Hunter-Kriterien für Serotonin-Toxizität (ST):

ST = Präsenz eines serotonergen Agens + eine Regel erfüllt.

1. spontaner Klonus

2. induzierbarer Klonus + (Agitation oder Hyperhidrosis)

3. okulärer Klonus + (Agitation oder Hyperhidrosis)

4. Tremor + Hyperreflexie

5. Rigor + Fieber >38 °C + (okulärer Klonus oder induzierbarer Klonus)

Realisation

First we built a standalone program based on Microsoft Access in collaboration with Stefan Kunz from the mathematical institute of the University of Berne. The complexity of the tasks and the high amount of data exceeded the capacity of this program quickly. It also became clear that an internet based program which is easily accessible and regularly updated would better cover the clinicians' needs.

Based on the gained experience from the prototype mediQ.ch was designed and subsequently implemented in close collaboration with Pierre Gumy from Pronaos GmbH by using open source software Zope and Plone. The platform is hosted by Stephan Göldi from Goeldi.com.

Discussion

Validation of mediQ.ch: Medical Thesis of A. Vieth, Mainz, 2008 (365)

One validation has been realized with data from 8/2007 in a medical thesis by Anna Vieth of the University of Mainz from 2008. She compared 4 German speaking drug interaction programs: PSIAC (www.psiac.de), mediQ (www.mediQ.ch), ifap (ifap index®KLINIK, on CD-ROM version 5/07 with data from ABDATA) and the interaction check from the Arznei-Telegramm (http://arznei-telegramm.de). In a Pubmed search she identified 40 clinically relevant drug interactions and 30 not clinically relevant drug interactions, examined them in each of the programs with the following criteria:

- Interaction pair in the program described?
- Mechanism of interaction described?
- Source reference of primary literature? (summary of product characteristic was not counted as such, neither were review articles or similar)
- Recommendations for the clinicians?
- Was the information to the interaction pair useful? E.g. was the relevance of the interaction adequately described? Was the information complete? And as most relevant, was the recommendation to the clinician helpful?

In 8/2007, the index time of the data capture for the medical thesis, the mediQ program had less than 4000 drug pairs described and stood at its beginning (although some evaluations concerning the program in general were made later when around 8000 drug combinations were described). End of 2009 almost 20'000 drug pairs are described. This meant that relevant information at the index period

was missing and sensitivity was rated as low (in 2009 all the examined drug pairs were described). The information found, however, was correct; the source information good with direct access to the abstracts of Pubmed, and as only program there was detailed information on the pharmacokinetics displayed as well. In 2007 only few recommendations to the clinicians were given, which was seen as drawback. This indeed was not an omission but at that time wanted. The mediQ team would only made risk estimations and the clinicians would then take the necessary measures. Three years experience with many different users of the mediQ program and the critique of A. Vieht, showed however, that many clinicians, especially young doctors or e.g. psychiatrists who rarely prescribe medication "cocktails" needed recommendations on what to do, and if necessary and possible, wished to get examples of alternative medications.

At the index period the user- friendliness was rated as suboptimal since not the whole information was displayed on the first page of the results. This might seem a disadvantage when only asking for the combination of 2 drugs; however, is a clear advantage when asking for more complex combinations, where mediQ in a graphical overview shows the available information with a first rating of the clinical relevance. This graphical display has been realised after the index period of 8/2007.

If the same validation would have been realised in 2009 or 2010, the mediQ program would have been rated high which according a personal communication of Christoph Hiemke from Mainz will be shown in a second thesis by Martina Hahn "Vermeidung von Interaktionen in der Psychopharmakotherapie".

Research project by Stefan Russmann et al, Zürich, 2009 - 2011

A research project on the clinical utility of clinical information programs such as mediQ and Theraopt/ ID PHARMA CHECK® has been performed by the Clinical Pharmacology Unit of the University of Zurich (Stefan Russman et al). They have filled in AMSP prescription data of almost 85'000 psychiatric in-patients in the mediQ-program and checked for the number and nature of alerts generated. A first manuscript on the results has been submitted in spring 2011. In a second step they plan to repeat this analysis with the medication of ADR cases of the AMSP project.

Comparison with other drug interaction programs

The use of dedicated software system in medical practice allows integrating automatic interaction checks directly into the work flow of the physician. These drug-interactions-checker-programs warn of the potential risks, give the level of significance of the interaction (major, moderate or minor), and in certain cases, provide the recommended course of action to manage the interaction.

However, conventional databases have a number of shortcomings, including:

- They are often empirical only, i.e. based on collections of clinical observations whose underlying causes usually are explained ex-post. This means that new drugs are not assessed, and that new biochemical and genetic information are not integrated.
- They assess interactions on the level of effectors classes of drugs, e.g. SSRIs, antipsychotics, etceteras. This can lead to dangerous misjudgements if combinations of drugs with an atypical compound included are assessed, e.g. a combination of clozapine and citalopram or clozapine with fluvoxamine is both rated as moderate interaction; however, fluvoxamine-clozapine can raise the plasma concentration of clozapine 10times and more, citalopram will probably have no effect at all.
- Novel concepts like the use of genetic profiling to stratify patients are not integrated in the data sets. The potential of pharmacogenetics for the risk-assessment of drug-drug interactions is foregone.
- Important information on diet or recreational drugs is not included.
- They are often based on linear ("conventional") texts and do not use the capabilities of electronic texts (such as hyperlinks inside the text, or links to references). These data sets tend to be heavy and cumbersome to use.

Some of the above mentioned tools are available online or are downloadable as personal digital assistants on Black Berry or iPhone. In many cases, though very interactive and with the promise of regular up-dated information, there is no knowledge about the source of the information which may pose an error risk.

Furthermore, with the exception of Genelex (www.genemedrx.com), pharmacogenetics and information on diet and recreational drugs are not integrated in the tools and new information-sets are very difficult to integrate without reprogramming the tools.

The analysis in table 22 is based on the 5 programs: Micromedex (Thomson Micromedex, Thomson Reuters 1974-2010: www.thomsonhc.com **)**, Genelex (www.genemedrx.com), Pharmavista (www.pharmavista.net), mediQ (www.mediQ.ch), PSIAC (www.psiac.de) which all reference the source of their information.

Table 22: Comparison of 5 drug interaction programs (Micromedex, Genelex, Pharmavista, mediQ, PSIAC). pk = pharmacokinetic

abilities	YES	Partly	NO
Risk estimation of combination >2drugs		Genelex, mediQ	Micromedex, Pharmavista, PSIAC
Pharmacokinetic information	Genelex, mediQ	PSIAC, Micromedex, Pharmavista	
Pharmacodynamic information	Micromedex, Pharmavista, mediQ, PSIAC	Genelex	
Side effect profile	Micromedex, Pharmavista, mediQ, PSIAC	Genelex	
Pharmacogenetics	Genelex, mediQ		Micromedex, Pharmavista, PSIAC
Age, gender, co-morbidities			None, sometimes in the text a warning
Diet/lifestyle	Genelex, mediQ, PSIAC		Micromedex, Pharmavista
Recommendations to the clinician	Micromedex, Pharmavista, mediQ, PSIAC		Genelex
Risk estimation without clinical cases	mediQ, PSIAC	Genelex pk, Micromedex/ Pharmavista class effects	

A broader overview is given in the table 23; it compares mediQ and the following databases/drug interaction programs on different features:

- e-mediat / ABDATA databases: today the most used interaction databases used in German language clinical decision support systems. Its origin comes from the “Deutsche Apothekerverband” (www.dimdi.de)
- Pharmavista (www.pharmavista.net): a standalone interaction program based on ABDATA
- Medical Letter's Adverse DID (http://secure.medicalletter.org): this program is currently overhauled and is expected to be available with more features mid of 2011.
- Micromedex (Thomson Micromedex, Thomson Reuters 1974-2010: www.thomsonhc.com **)**
- PSIAC (www.psiac.de)
- Genelex (www.genemedrx.com)
- Epocrates (www.epocrates.com)
- PEPID (www.pepid.com)
- Davis Drug Guide (www.unboundmedicine.com)
- Lexicomp (www.lexi.com)
- Skyscape (www.skyscape.com)
- Drugs.com (www.drugs.com)

Table 23: Comparison of electronic information sources on drug interactions

Program	group adressed	information sources referenced	More than class effects	Pharmaco-genetic information	More than only linear text	Risk estimation of combination > 2 drugs	Pharmaco-kinetic information	Pharmaco-dynamic information	Side effect profile	Age, Gender, comorbidities	Diet / Liefestyle	Recommen-dations to clinician	Risk estimation without clinical cases
emediat/ABDATA Data collection	health prof	YES	NO	NO	NO	NO	PARTLY	YES	YES	PARTLY	NO	YES	YES
Pharmavista (data from e-mediat)	health prof	YES	NO	NO	NO	NO	PARTLY	YES	YES	NO	NO	YES	PARTLY
Medical Letter's Adverse DID	health prof	YES	PARTLY	NO	NO	NO	PARTLY	YES	PARTLY	NO	YES	YES	PARTLY
Micromedex	health prof	YES	YES	NO	NO	NO	PARTLY	YES	YES	NO	NO	YES	PARTLY
PSIAC (only psychiatry)	health prof	YES	YES	NO	NO	NO	PARTLY	YES	YES	NO	YES	YES	YES
Genelex	health prof	YES	YES	YES	YES	PARTLY	YES	PARTLY	PARTLY	NO	YES	NO	PARTLY
Epocrates Rx	health prof	NO	YES	NO	NO	NO	no info	no info	YES	YES	PARTLY	PARTLY	YES
PEPID	health prof	NO	YES	NO	YES	NO	PARTLY	YES	YES	NO	YES	YES	YES
DavisDrugGuide	health prof	NO	PARTLY	NO	NO	NO	no info	no info	no info	YES	PARTLY	YES	YES
Lexi-Comp	health prof	NO	no info	NO	NO	NO	no info	no info	no info	no info	no info	PARTLY	YES
Skyscape (data from Medical letter)	Patients: simple warning	NO	no info	NO	NO	NO	no info	no info	no info	NO	no info	NO	no info
Drugs.com	Patients: with explanations	NO	PARTLY	NO	NO	NO	no info	no info	YES	NO	PARTLY	YES	YES
mediQ.ch	**health prof**	YES	YES	YES	YES	PARTLY	YES	YES	YES	PARTLY	YES	YES	YES

Other authors looked from a different angle to several drug interaction programs and rated e.g. time to retrieve the relevant information, accessibility through mobile devices, or studied the “signal to noise ratio”, the problem of too many alerts leading to ignoring given alerts (361;366-374). The risk of ignoring the alerts of an interaction program because there are too many alerts can lead to build in filters for seeing only the interaction bearing a major risk. However, this can be misleading by overlooking an accumulation of equal or similar effects in a drug combination with more than 2 drugs. Better than filters are short graphical overviews where the clinician gets on first eyesight an impression of the interaction potential, and from there he or she can decide to go more into details. Further points of critique were that patient factors are not taken into account, that there are no standards of how clinical relevance is rated and in mobile devices but also some pharmacy programs

the quality of information seems to be at times suboptimal, partly because no automatic updates were available.

Drug interactions programs: clinician's needs and program's realities

Electronic programs to aid the physician with prescribing drugs can be valuable tools for the safety of patients and may become integrated in the quality assurance in medical practice. Drug interactions programs give risk estimations of drug combinations, which dependent on the factors considered – also patient characteristics - will be more or less accurate. Currently there exists no program which is able to predict the outcome of a drug (combination) treatment, the interplay of a multitude of influencing variables being too complex. This is also true for mediQ.ch.

Clinicians wish a precise risk prediction of a drug combination therapy, not only for the combination of two drugs but also for more. The program should also take into account patient factors such as renal or hepatic insufficiencies, age, gender, illnesses, pharmacogenetic factors, diet, and lifestyle.

However, the complexity of the interplay between drugs and the patient variables represent a seemingly insurmountable challenge at least as of today. One can imagine that new mathematical models to combine all these variables will allow a more precise outcome prediction one day. The clinician has to be aware of the advantages but also of the shortcomings of today's drug interaction programs.

Financial and legal aspects

As of today clinicians are not obliged to consult drug information programs but they have the duty to care and to prevent harm. Being knowledgeable of the interaction risk of a drug combination might also legally belong to this duty. In Switzerland there is no court case known, where a doctor has been convicted because he or she has overlooked a drug interaction risk (personal communication with Ursula Eggenberger Stöckli, Bern, lic. Iur. and dipl. Pharm.). It will be interesting to see how this will change when electronic prescribing with an integrated drug interaction checker will become part of standard care. The situation in the USA is different. There exist lawyers who are specialised in medical malpractice and some are specialised in injuries as consequence of drug interactions. Courts might view a drug interaction due to a pharmacokinetic interaction as excessive dosing or as failure to appropriately monitor plasma levels (375); some case of death or permanent injury resulted in high financial damage compensations, as e.g. in a case 1999 in Oregon where ciprofloxacin (strong CYP1A2 inhibitor) and theophylline (CYP1A2 substrate) were co-prescribed resulting in permanent brain damage because of theophylline intoxication (375).

Another legal aspect is the liability of the authors of drug information programs. Users must exercise their independent professional judgment and should always consider the latest manufacturer's legal

information. A drug interaction or information program has to be used as a clinical decision support system, it cannot be more. Nevertheless the authors of drug information programs should be aware of their obligation to offer the most accurate information possible since clinicians will rely on their information and patients' wellbeing might depend on it. This also implies regular updates on one hand and dated information on the other hand.

Costs play a role in the decision of doctors and pharmacist to consult drug information programs but costs are also important in building up and maintain high quality programs. Nowadays cost differences are high. Some programs such as www.drugs.com are freely accessible and are paid by advertisement. Others are expensive such as Micromedex Drug Reax where a single user licence for a clinician costs approximately 5000 Euros, Pharmavista costs around 650 CHF, more often the costs sum up to 100-250 Euros per single user (PSIAC; Genelex, mediQ.ch...). For the moment, these programs are competing with each other, and each of them has high salary costs for qualified personnel to pay. In the case of mediQ.ch, salaries are paid by the clinic Königsfelden and the income generated by the mediQ.ch licences, no pharmaceutical sponsoring is allowed.

Collaboration could be useful, as could be a governmental support to make high quality programs available to all.

Future Challenges for mediQ.ch

Challenges for the mediQ.ch program will be to become integrated in the (electronic) prescribing process, to keep all the information regularly updated in order to maintain a high quality database and to provide continuously clinically relevant information and recommendations to the clinician. The risk of over-alerting must be kept in mind. When a clinician considers the information too abundant and not enough relevant, he or she will discard the alerts and the interaction program loses its purpose. A multi-lingual program would serve more persons and would on the long-term become more cost-effective. mediQ.ch has a structure which would allow a multi-lingual approach. If enough financial resources are to be found a translation in other languages is probable.

Interfaces to clinical information systems are currently being programmed and first tests seem promising. Since most clinical information systems already have a more general drug interaction program such as Pharmavista integrated, the problem of too much and sometimes contradicting information will have to be addressed.

8 Pharmacovigilance in Psychiatry: case studies

Case reports are important documentations of uncommon events – clinical studies are most often not large enough to document rare adverse drug reactions therefore post marketing surveillance is essential – case reports can illustrate theoretical assumptions and often serve didactic purposes as well.

They are of particular interest as "real life" documentation of drug effects in patients where only studies in healthy volunteers or clinical studies with stringent in- and exclusion criteria exist.

However, case reports can only be used for signal generation. False conclusions can be drawn when unexpected events are wrongly interpreted, sometimes because not all pieces of the puzzle are known. E.g. a drug B is added to a drug A, plasma levels of drug A raise: obvious conclusion: drug B inhibits drug metabolism of A – but: shortly before adding drug B a drug C had been stopped, this drug C is an inducer of the metabolism of drug A and that fact has been overlooked. A cause to effect relationship needs pharmaco-epidemiological studies.

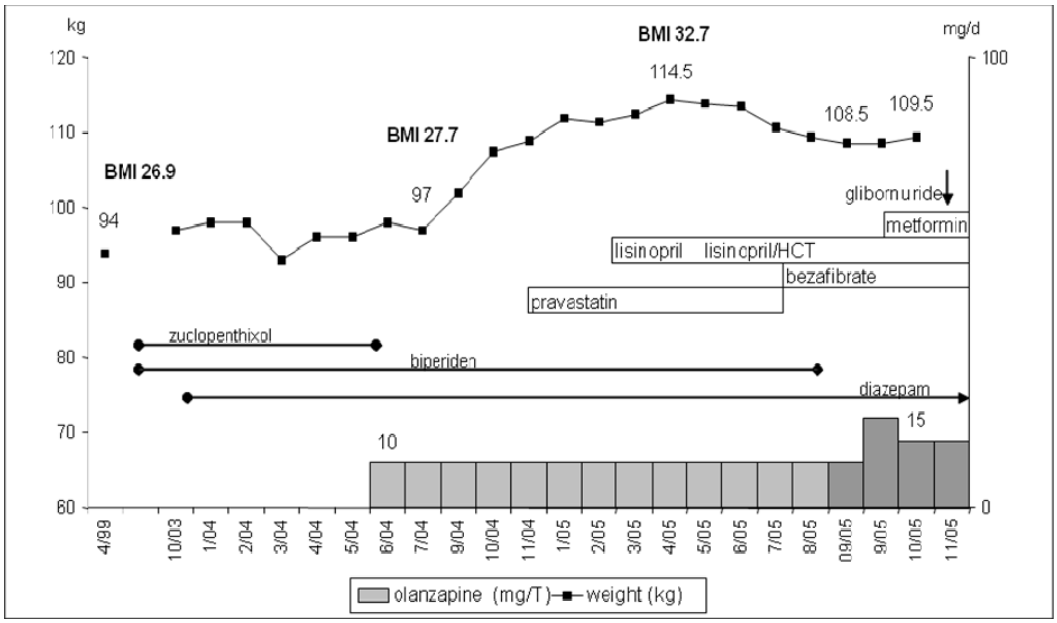
The following case (series) reports are examples; a brief summary of their publication is given here.

Metabolic syndrome associated to clozapine and olanzapine (266)

A case-series of three chronic schizophrenic patients are presented who responded only to clozapine or olanzapine but suffered from massive weight gain and developed a (partial or full) metabolic syndrome. A change of medication was not an option since treatment changes resulted in exacerbation of their schizophrenic symptomatology. Therefore pharmacological and non-pharmacological strategies to counteract the weight gain under these antipsychotics were searched.

The first case describes a 34 years old man who developed tardive dyskinesia under zuclopenthixol and was therefore changed to olanzapine. He had a body mass index (BMI) of 29 before first intake of olanzapine 10mg/d, within 10 months of this treatment his BMI raised to over 35 and he developed a full metabolic syndrome (fig. 33). As counteractive measure a galenic change to dispersible olanzapine tablets and diet and fitness counselling was installed. With this the patient lost 6kg within 4 months.

Figure 33: Time course of weight and metabolic changes of case A during treatment with olanzapine



The second case describes a 33 years old woman who gained 44 kg (76 kg to 120kg, BMI 37.5) within 5 years of clozapine treatment, most of the time in combination with valproate. This massive weight gain corresponding to 58% of her original weight caused walking problems with pain and urinary stress incontinence. She began to develop a metabolic syndrome with lipid changes and partially high blood pressure. Change to another pharmacotherapy was several times tried but always failed.

The third case describes a 33 years old male patient who almost doubled his weight (67 to 128kg) under clozapine treatment in less than 4 years. As counteractive measure combinations with topiramate (-8 kg) and later with fluvoxamine (-18kg) helped to lose weight. During his massive weight gain his blood pressure was high and lipids and glucose parameter were suboptimal which partially normalised following weight loss.

Discussion:

Weight gain is a common but often underestimated health problem with many antipsychotics, most important with clozapine and olanzapine. Sometimes, especially in the cases of massive weight gain, it is accompanied by a metabolic syndrome. The reader should get sensitised to the clinical impact of such weight gain and learn strategies to prevent or minimize it.

Electric sensations: neglected symptom of escitalopram discontinuation (376)

Treatment with citalopram (20 mg/d) was initiated in a depressive patient, which was soon replaced by escitalopram (10 mg/d, as monotherapy), the pharmacological active enantiomer of citalopram. Responding well, the patient reduced after 2 months first this dose to 5 mg/d and three weeks later stopped treatment completely. About a week later, she experienced electric shock-like sensations or flashes which were also visual, lasting for about one second each. This was followed by a phase of spatial disorientation that lasted for about 30 seconds and was experienced as highly unpleasant and frightening. The sensations were only felt in an upright position; the patient has no history of loss of tonicity. These episodes occurred up to three times a day over a period of two weeks. Prodromal symptoms or specific triggers were not reported. While the patient was on citalopram/escitalopram, no side-effects were observed. About 6 weeks later her depressive symptoms returned, resulting in another therapy with escitalopram (10 mg). After feeling better she stopped again taking her antidepressant escitalopram and experienced another withdrawal syndrome with the same symptoms as the first time, although this time they were less intense.

Discussion:

This case describes in detail - including dechallenge and rechallenge of the drug treatment - the symptomatology of an SSRI withdrawal syndrome after cessation of an escitalopram treatment. This was then the first case about a withdrawal syndrome published with this specific SSRI.

Smoking not nicotine (377)

A 50-year-old female inpatient, a heavy smoker, was treated for organic psychotic disorder and epilepsy with clozapine 75 mg/day, fluvoxamine 150 mg/day and valproate 1.5 g/day. After smoking cessation, because of bronchitis with elevated CRP, and supported by nicotine substitution (transdermal patch), her serum level of the CYP1A2 substrate clozapine increased by factor 2, while dosage and co-medication with valproate and the strong CYP1A2 inhibitor fluvoxamine stayed the same. CYP1A2 is induced by polycyclic aromatic hydrocarbons (not nicotine) in tobacco smoke and probably inhibited by infect related cytokines. The lack of induction (smoking cessation) and transient inhibition of CYP1A2 (bronchitis) caused a marked increase of clozapine level, even in presence of the strong CYP1A2 inhibitor fluvoxamine. Smoking cessation and infections with elevated CRP while on clozapine therapy require evaluation of dose reduction, monitoring of serum levels and screening

for signs of overdosing. It is important to note that an increase of clozapine level is not prevented by nicotine substitution (cf. above).

Discussion:

This case illustrates how co-medication and patient factors can affect the plasma concentration of a drug with a relatively narrow therapeutic index. CYP450 modulation (induction and inhibition) are discussed as well as the fact that clozapine has not a linear concentration-dose relationship.

Clonus, hyperreflexia and agitation in a patient with increased fluvoxamine plasma level: signs of serotonin toxicity (378)

This case report describes a 28 years old woman with mild mental retardation and significant behavioral impairment resulting from anxiety and obsessive-compulsive symptoms. She was admitted to the psychiatric clinic Königsfelden. Neither her neurological examination including MRI and EEG nor laboratory values revealed any abnormalities. Her treatment with 200mg/d fluvoxamine was stepwise increased to 400mg/d and valproate 2000mg/d was added. She showed signs of sedation and her initially light tremor under fluvoxamine 200mg/d became more severe. She also complained about cramps in her legs. Neurological examinations showed a middle frequent tremor of all extremities, a marked hyperreflexia of the legs and an Achilles tendon reflex clonus of 3-5 contractions. Fever, tachycardia, excessive sweatening, and high blood pressure were not observed. Trough plasma levels were for fluvoxamine 620ng/ml (ref. 150-300ng/ml) and for valproate 154 µg/ml (ref. 50-100 µg/ml) (41). Valproate was stopped and fluvoxamine stepwise reduced and with that the neuromuscular hyper-excitability disappeared gradually, after stopping fluvoxamine no hyperreflexia was noted anymore.

Adverse drug reactions as a result of increased central serotonin levels are best described by the term ”serotonin toxicity” (ST) implicating concentration dependent effects. ST is characterized by the triad of neuromuscular signs, changes of mental status and autonomic symptoms, clonus being a key symptom. Severe ST at therapeutic doses mainly occurs, when inhibitors of monoamine oxidase type A (MAOI-A) are combined with serotonin reuptake inhibitors (SRI) of any kind. In Switzerland irreversible nonselective MAOI antidepressants are not marketed. Nevertheless a number of drugs have MAO-A inhibiting properties: moclobemide (reversible, selective MAOI-A) and the antibiotics linezolid (reversible, nonselective) and isoniazid (irreversible, nonselective). The antiparkinson drug selegiline (irreversible, selective MAOI-B) loses MAO selectivity at higher doses, an effect which also

has been shown for rasagiline. A number of drugs and combinations can - even in absence of MAOI - precipitate mild to moderate ST, a condition which can be difficult to recognize and impair compliance: (S)SRI, tricyclic and ”dual action” antidepressants (and sibutramine), serotonin precursors, some opioids, stimulants and antihistamines. Treatment options are dose reduction (mild ST), treatment discontinuation, benzodiazepines and 5-HT2A-antagonists (severe ST).

Discussion:

With the description of this case under a high dosed SSRI, the symptomatology of serotonin toxicity which can comprise only some typical serotonergic symptoms up to a full serotonin syndrome is presented. Diagnostic criteria as well as precipitative agents and counter measures are discussed.

Adverse drug reactions following non-response in a depressed patient with CYP2D6 deficiency and low CYP 3A4/5 activity: a pharmacovigilance case report (379)

A 47-year-old male taxi driver experienced multiple adverse drug reactions during therapy with high-dose clomipramine and quetiapine for major depressive disorder, after having been unsuccessfully treated with adequate doses of mirtazapine and venlafaxine. Drug serum concentrations of clomipramine and quetiapine were unusually high. Pharmacogenetic testing showed a poor metaboliser status for CYP2D6, low CYP3A4/5 activity and normal CYP2C19 genotype. After reduction of the clomipramine dose and discontinuation of quetiapine, all ADRs subsided except for the increase in liver enzymes. The latter improved but did not normalize completely, even months later, possibly due to concomitant cholelithiasis.

Discussion:

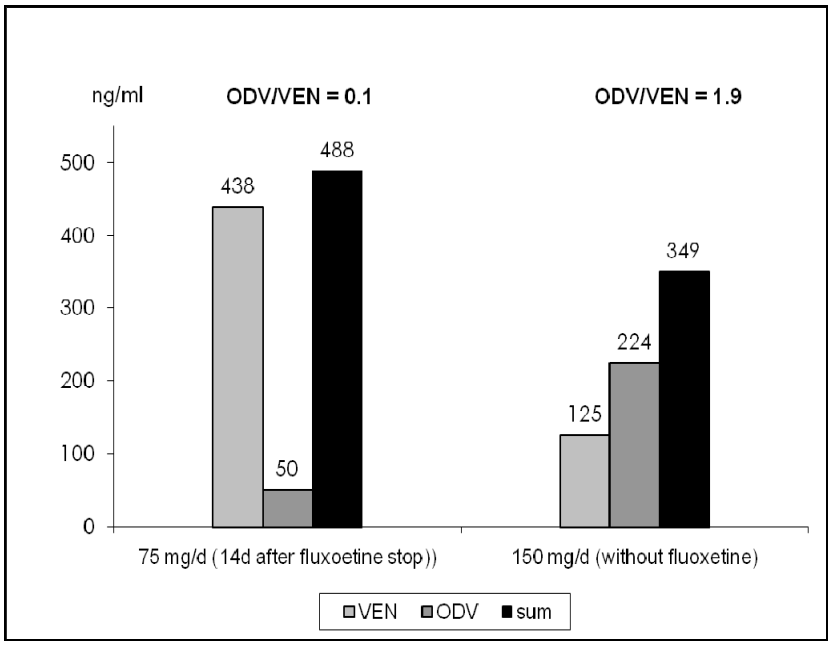
This patient showed an unusual combination of CYP2D6 *4/*6 and a probably genetically determined very low CYP3A activity comparable to inhibition by ketoconazole (a very strong CYP3A-inhibtor). Clinical effects, preventive and correcting measures are discussed, Therapeutic Drug Monitoring - re- or better proactive - being given special attention.

Misinterpretation of a venlafaxine blood level (380)

A 42 years old depressed female patient was admitted to the psychiatric clinic Königsfelden pre-treated with an SSRI. Her treatment was changed to a regimen of 75mg/d venlafaxine, mirtazapine 15mg/d and valproate 1000mg/d. Blood levels of venlafaxine (V) and its active metabolite O-

desmethylvenlafaxine (ODV) were measured because she suffered of an adverse event. The results showed an unusual high concentration/dose (488ng/ml V + ODV with a venlafaxine dose of 75mg/d) and a very low metabolic ratio ODV/V of 0.1. Subsequently the dose was lowered to 37.5mg/d which finally led to a depression relapse. Further investigations revealed that the low metabolic ratio was due to a CYP2D6 inhibition by the formerly taken fluoxetine. Interestingly, two weeks after the last intake of fluoxetine 40mg the plasma levels (determined from a frozen serum probe) were still in the recommended therapeutic range: fluoxetine 136 ng/ml, norfluoxetine 136 ng/ml, ref. for the sum 120-300 ng/ml. This unusual metabolic rate is also seen in CYP2D6 poor metabolisers, but this patient was in an earlier hospitalisation genotyped and genetically (CYP2D6*1/*1) a CYP2D6 EM. ”After readmission (10 weeks later) the patient’s venlafaxine doses was increased to 150mg/d, the plasma levels of venlafaxine plus O-desmethylvenlafaxine were with 349ng/ml lower than before and the metabolic ratio ODV/V was now 1.9 (fig. 34).

Figure 34: metabolic ratio of O-desmethylvenlafaxine (ODV)/venlafaxine (V) with and without influence of the co-medication fluoxetine



Discussion:

This case illustrates how discontinuation of medication with a long half life can continue its effects including CYP450 modulation. The reader is invited to thoroughly judge the patient’s medication history. Further possible interpretations of the metabolic ratio including pharmacogenetics are discussed.

Blood levels of extended-release quetiapine: beware of misinterpretation (48)

A patient treated with extended-release quetiapine showed an unusual high blood level (1204 ng/ml, therapeutic range 70-170 ng/ml). On the basis of this example and a further three cases the specific pharmacokinetics of extended-release quetiapine and problems concerning the interpretation of its blood levels are discussed. Plasma levels measured 12-16 hours after last drug intake are valid for quetiapine immediate but not for quetiapine extended release. Trough levels are not yet reached in the latter case. Plasma levels measured 10 hours after drug intake were about 5 times higher than levels measured after 20 hours.

Discussion:

In many psychiatric clinics blood drawing for plasma level analysis is only done in the morning before first drug intake. Quetiapine is a drug which is mostly prescribed as an evening or night dose and therefore plasma levels are taken after about 12 hours. This common practice is acceptable for many psychopharmacological agents but as shown in the case series not for quetiapine (and probably other) extended release medication where trough levels are better taken after 20-24 hours.

Combination of clozapine with fluvoxamine (69;331)

A 48-year-old smoker with chronic schizophrenia was treated with a very high dose of clozapine (1200 mg/day) in order to obtain plasma levels within the recommended range of 350-600 ng/ml and to attain a therapeutic response (the patient was phenotyped as a rapid CYP1A2 metaboliser due to a high smoking-related CYP1A2 inducibility). After 2 years of this high-dose treatment he experienced several grand mal epileptic attacks. It was hypothesized that the use of such a high dose of clozapine produced high peak plasma levels, which could be a risk factor for his epileptic attacks. Treatment was therefore changed to a combination of a strong CYP1A2 inhibitor, i.e. fluvoxamine (150 mg/day) + 125 mg/day clozapine, in order to decrease peak plasma levels, while maintaining the trough levels. The therapeutic response remained stable and trough plasma levels stayed within the same range as those observed with high dose clozapine treatment. However, the patient did not experience any further epileptic fits.

Discussion:

Adding fluvoxamine to clozapine treatment can increase plasma levels of clozapine by up to 10-fold (69;315;381-383), which can be highly effective (69;315;382-384) but is not without risk of intoxication (drowsiness, epileptic seizures, delirium and cardiac problems). Regular TDM control during the treatment switching period is necessary, as well as an immediate adaptation of the clozapine

dose. It is important to remember that the extent of the inhibitory effect is dependent on the baseline metabolic activity of the enzyme to be blocked and on the dose of the blocking agent (320;385). Another effect will be a shift of the concentration relationship between the parent compound clozapine and the metabolite norclozapine, which might represent a certain advantage in the tolerability of the treatment since norclozapine seems to be more sedative. It has also been postulated that combination therapy with fluvoxamine causes less weight gain than monotherapy with clozapine alone (315).

Drug interaction leading to loss of therapeutic effect

A 38-year-old patient with a history of drug abuse, psychotic episodes and human immunodeficiency virus (HIV) infection was admitted to hospital in an aggressive psychotic state. Treatment with a high dose of zuclopenthixol 400 mg depot, diazepam 60 mg/day and methadone 90 mg/day improved his condition. Because of his HIV-infection he subsequently received lamivudine and efavirenz. Five days after starting the new HIV treatment the patient deteriorated rapidly and became highly aggressive again. TDM of methadone showed a decrease in the methadone concentration to 55% of the baseline value, resulting in methadone withdrawal symptoms. HIV treatment with efavirenz was stopped and the patient recovered after about one week; the methadone plasma levels concomitantly returned to baseline values.

Discussion:

The described case (unpublished observation) and similar published cases (386;387) demonstrate a drug metabolism-inducing effect leading to methadone withdrawal. Efavirenz (388) and nevirapine (389) are strong inducers of CYP3A4, a major pathway in the metabolism of methadone. A prospective stepwise increase of the methadone dose (in general about 25%) together with multiple daily dosing and TDM are recommended.

Interaction with concomitant illness

A 54-year-old obese diabetic non-smoking patient with chronic schizophrenia was treated with a stable clozapine dose (and stable plasma levels in the therapeutic range) for several months. She became drowsy and partly disoriented during a symptom free pericarditis. Her clozapine plasma levels were at 250% of the recorded levels before the pericarditis without any change in medication dose.

Discussion:

Similar cases, mainly with respiratory infections like pneumonia have been described (390;391). The clinicians should be aware of potential clozapine intoxication in presence of a severe inflammatory process, measure clozapine plasma levels and adapt accordingly the dose. This might be valid for other drugs which are mainly metabolised by CYP1A2 such as olanzapine and theophilline.

Conclusion

Although case reports cannot be generalized they are issued from clinical "real life". Sometimes they illustrate what previously existed only as a theoretical hypothesis (pharmacodynamic interaction) or as a prediction from in vitro data (e.g. pharmacokinetic interaction). Sometimes their events are completely unexpected and further systematic research with pharmaco-epidemiological tools can confirm or refute an unknown adverse drug reaction. Sometimes they also show how normally well tolerated drug combinations can be harmful in vulnerable patients with risk factors such as low renal function, pharmacogenetic mutations, or an electrolyte abnormality.

Case reports containing information on confirmed or suspected drug interactions are useful in the estimation of drug interaction risks and are referenced in the drug interaction program mediQ.ch. Often this is the only published clinical information available.

9 Concluding remarks and outlook

During the past decade, Therapeutic Drug Monitoring, drug interaction checking programmes and pharmacogenetic tests have become more integrated in the clinician's prescription process. A major step forward was the publication of the TDM consensus paper for psychiatry in 2004 (41), which provides guidelines for plasma level analysis and reference plasma levels. Thanks to the experiences made in pharmacovigilance programmes such as AMSP, where drug plasma levels have become an important factor to assess causality, and from published case reports linking observed ADRs or therapeutic failures to unusual and/or unexpected plasma levels, awareness is increasing. In 2011, updated and extended TDM guidelines will be published for therapeutic and dose related reference levels and including, for the first time, the notion of an alert plasma level, since the upper value of the recommended levels is often misinterpreted as the toxic threshold. This can, in some cases, lead to unnecessary dose adaptations and subsequent treatment failure.

Although in 2011 clinicians are more familiar with therapeutic drug monitoring than they were 10 years ago, efforts to teach the correct handling and interpretation of drug plasma levels to ensure meaningful results must continue. At the Psychiatric Clinic Königsfelden, it seems that involuntary intoxications due to unrecognised drug interactions or pharmacogenetic vulnerability are being detected earlier and are becoming less common. To confirm this assumption, we plan to compare drug plasma level results and drug combination data from 2000-2002 with those of 2009-2011. However, the introduction of regular TDM is not the only factor that has contributed to a lower number of harmful drug interactions: the introduction of our drug interaction programme mediQ.ch in the autumn of 2006 has also had a significant impact, as did the introduction of our test algorithm TDM plus, following which pharmacogenetic test results explained a number of unusual plasma levels.

How did the work in connection with this thesis contribute to improve drug safety for the psychiatric patient?

Psychiatric patients are often exposed to polypharmacy of different psychotropic and somatic drugs their whole life, whereby the latter are sometimes administered to combat adverse effects of the first. The chronic use of drugs bears extra risks (e.g. weight gain and metabolic syndrome with modern, tardive dyskinesia, in the case of classic antipsychotics), risks which are hardly detected before a drug reaches the market. In phases of symptom exacerbation, the psychiatric patient might be medicated against his or her will in an emergency situation, be given high doses of different psychotropic drugs while being unable to communicate negative side effects. It is important to recognize and understand

ADR risk factors by undertaking clinical trials, case-control studies and by learning from well-analysed case reports. It is necessary to raise awareness of these risks and to offer a tool that helps clinicians assess risks quickly.

AMSP is a pharmacovigilance programme which helps finding risk factors and generates safety signals for new ADR in psychiatric patients under naturalistic conditions. It represents an instrument of quality assurance and continuous education of medical doctors in psychiatric wards. By discussing the cases of the AMSP+ study, TDM and pharmacogenetics have been introduced to members of the AMSP programme during the different case conferences. In the course of the last decade, TDM has become a valuable tool for causality assessment of SADR, and pharmacogenetic variation is now generally recognized as a risk factor. The AMSP+ study was able to confirm that high drug plasma levels represent a risk factor for experiencing an SADR with a statistically significant odds ratio of 3.5 for drug plasma levels >120% of the upper recommended reference level. AMSP data show that the hospitalisation period doubles when a psychiatric patient experiences a serious adverse reaction. If drug plasma levels are measured at an earlier stage, we are able to prevent a number of SADR. We can furthermore guide the clinician in his or her choice of reducing the dose or changing the drug. However, for economical reasons we cannot recommend routine TDM. The indications for TDM given in the consensus guidelines should be followed.

The situation concerning pharmacogenetic tests is more complex. On the one hand, more research, such as our clinical trials with mirtazapine and clozapine, is needed to understand which genotypes influence the effects of which drugs. On the other hand, we have to understand the nature of genotype-related risks. We were able to show that the case-control study design is appropriate to identify rare events such as SADR and their risk factors. A state-of-the-art paper on pharmacogenetic studies also mentions the case-control design as suitable for studying SADR and pharmacogenetics (349). We will continue our research into risk factors of SADR in psychiatry and will analyse a larger case-control group for the impact of high plasma levels and more *CYP450,* and possibly *ABCB1,* genotypes.

Based on our experience gained during the AMSP+ study and on the fact that we have only sparse data to support routine TDM and/or pharmacogenetic testing, we developed the test algorithm “TDM plus”. This algorithm is followed at the Psychiatric Clinic Königsfelden and has been instrumental in avoiding unnecessary pharmacogenetic tests. It has also become part of many continuing medical education lectures in and outside Switzerland. It is our firm belief that TDM and pharmacogenetic tests are valuable instruments of pharmacovigilance, not only in the causality assessment of ADR, but also in limiting harmful effects and in preventing them in future treatments.

It is not enough to study and publish pharmacological characteristics of drugs or ADR related risk factors, this knowledge must be available to the treating physician at the moment of prescribing the

medication for the patient. A variety of electronic drug information programmes have become available, with the most successful programmes being accessible through the internet, which allows information retrieval at any time from almost every location. Future prescribing will most probably be done electronically on a handheld device.

mediQ.ch has taken another approach than most of the current drug interaction programmes by being designed “bottom up”, meaning that risk assessment is based on the clinical and pharmacological data available for the combination of two specific drugs. It allows easy updating and combining interaction risks with other factors such as specific genotypes, diet, lifestyle or co-morbidity. Medical professionals from more than 130 hospitals, and 400 independent physicians in the German-speaking countries, consult this programme regularly. Their feedback is taken into account to fine-tune the programme to meet the users’ needs. mediQ.ch gives more detailed and more in-depth information than most other programmes. However, this detailed information rather meets the needs of hospital doctors and pharmacists than those of doctors or pharmacists in the field. One might consider developing a “light” version for them and adapting it for use with mobile devices such as smart phones or the I-Pad. This may also meet the needs of patients.

mediQ.ch will be developed further, ideally to cover all clinically significant interactions, although such a goal might seem ambitious considering the vast number of possible drug pairs. Each user has the possibility to request information about such drug pairs of a drug combination therapy that have not yet been described. Answers will be provided within 48 hours, and the missing information is added to the programme. All information has to be periodically re-examined and, if necessary, updated. To support this task, the programme has an automatically generated reminder for information that is older than 2 years.

Another challenge will be the integration of the drug interaction check of mediQ.ch into the electronic prescribing process and to make this large information database available in other languages. In general, more collaboration and consensus between different drug information providers should be attained and information from different sources combined, so that drug prescribing becomes safer. In order to avoid possible medication errors, several governmental bodies are considering guidelines for electronic prescription programmes which would include an interaction check. This may eventually lead to a certain level of harmonisation between the information from different providers.

Finally, has the target of avoiding high-risk drug combinations been reached? Are there fewer SADR due to drug interactions? A recent publication on AMSP data has shown that although polypharmacy has increased between 1993 and today, the number of high-risk combinations (as measured by mediQ.ch) has in fact decreased (392). This may indicate that high-risk combinations are avoided more often and replaced by low-risk drug combinations. Our impression based on the AMSP project is

that the severity of Type A ADR has decreased. Serotonin toxicity e.g. is being recognised early and a dramatic course is thus being prevented. Also, more targeted laboratory or ECG controls are carried out in high-risk drug combinations, which can prevent serious ADR. Very high drug plasma levels resulting from a pharmacokinetic drug interaction have also become less frequent.

Our hope

Increasing knowledge of drug characteristics, pharmacogenetics and environmental factors, together with more advanced and less costly laboratory and information technologies, will enable us to find a way to practice a (more) personalised medicine, ensure safer and better tolerated drug treatments and a higher therapy success rate.

10 Own Publications

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