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PHARMACOTHERAPY OF PATIENTS WITH SCHIZOPHRENIA LIVING IN NURSING HOMES

-Impact of genetic and environmental factors

Master thesis submitted to Department of Pharmacology,

Section of Biology, School of Pharmacy,

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for the degree candidata pharmaciae

Tore Haslemo

October 2004

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ACKNOWLEDGMENTS

Thanks to Per Håkon Eikeseth for initiating this project, providing patients to the study and for cooperation throughout this year.

Thanks to Lars Tanum and Helge Refsum for supervision, inspiration and help during the entire process.

Thanks to Linda Hårstad for performing all PCR-tests and to Hilde Lunde for HPLC-MS/MS analyses of blood samples.

Thanks to the staff at Department of Psychopharmacology for blood sampling, handling of study material and for useful help.

Thanks to Hege Christensen and Anders Åsberg for motivation, hints and helpful aid in the process.

Finally thanks to family and friends for support and interest.

Vinderen, 20. October 2004

Tore Haslemo

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ABBREVIATIONS

ADR	Adverse drug reactions
ATC	Anatomic therapeutic chemical
CI	Confidence Interval
CNS	Central nervous system
CYP	Cytochrome P450
DDD	Defined daily doses
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
ESI	Electro spray ionization
HPLC	High pressure liquid chromatography
ICD-10	Tenth Revision of the International Classification of Diseases
IM	Intermediate metabolizer
LLE	Liquid-liquid extraction
MS	Mass spectrometry
PAH	Polyaromatic hydrocarbons
PANSS	Positive and negative syndrome scale
PCR	Polymerase chain reaction
PK	Pharmacokinetic
PM	Poor metabolizer
SD	Standard deviation
SRS	Substrate recognition sites
SSRI	Selective serotonin reuptake inhibitors
TDM	Therapeutic drug monitoring
UM	Ultra rapid metabolizer
WHO	World Health Organization

ABSTRACT

Schizophrenia is characterized by early onset and chronic lapse in most of the patients. The suffering associated with schizophrenia is enormous – for patients, families, and in society at large. Pharmacotherapy is the mainstay of treatment, without which most psychosocial treatment would not be possible. However, substantial variety in efficacy as well as frequently reported side effects are problems often encountered with current antipsychotic treatment. Cytochrome P450 (CYP) enzymes are involved in the metabolism of most psychotropic drugs and characterized as the most important factor for individual variation to drug response.

This study investigated pharmacotherapy of schizophrenic inpatients in seven different nursing homes receiving long-term antipsychotic treatment. Impact of environmental- and genetic factors on serum concentration of antipsychotics was studied. CYP-genotyping of isoenzymes CYP2C9, CYP2C19 and CYP2D6 were performed for both a patient group (n=109) and a control group (n=136). Clinical data were collected from records and medicine cards. Serum concentrations (trough) were obtained for all antipsychotics used by the patients.

The patients were on average treated with 1.5 antipsychotics and 2.7 concomitant drugs. Our patient group received a total of 2.2 defined daily doses (DDD) of antipsychotics compared to an average of 1.2 DDD reported in a study of psychiatric hospital inpatients. The measured genotypes were not significantly different between the patient- and control group. Only one of the patients had been genotyped prior to the project. Patients being poor metabolizers had higher serum concentrations and patients being ultra rapid metabolizers had decreased serum concentrations from a given dose. CYP2D6 intermediate-metabolizer genotype appeared to be important in some patients, especially when other factors (e.g. environmental, polypharmacy) were present. Low doses of CYP2D6 inhibitors appeared to be of minor importance as long as it was the only factor influencing CYP activity. Use of CYP inducers led to decreased serum concentrations. Smoking did significantly decrease serum concentrations of CYP1A2 substrates and the induction seemed to be similar in heavy compared to light smokers.

Pharmacotherapy in patients suffering from chronic schizophrenia is characterized by polypharmacy and somewhat high doses of antipsychotics. Both genetic and environmental factors appear to influence the serum concentration. CYP-genotyping combined with therapeutic drug monitoring can be an important tool in individualization of drug regimes. Reassessing the pharmacotherapy of some of these patients might lead to an improvement of their current treatment.

1 INTRODUCTION

1.1 Schizophrenia

Schizophrenia is defined by World Health Organization (WHO): "A severe emotional disorder of psychotic depth characteristically marked by a retreat from reality with delusion formation, hallucinations, emotional disharmony, and regressive behaviour".

The essential features of schizophrenia are a mixture of characteristic signs associated with marked social and or occupational dysfunction. The condition is not caused by direct physiological effects of a substance or a general medical condition. The characteristic symptoms involve a range of cognitive and emotional dysfunctions: Perception, language and communication, drive, affect, attention, behavioural monitoring and fluency and productivity of thought and speech. No single symptom is equivalent with schizophrenia; the diagnosis involves the recognition of a constellation of signs and symptoms associated with impaired occupational or social functioning. These can be divided into two broad categories – positive symptoms (excess normal functions) and negative symptoms (loss of normal functions). Positive symptoms include delusions, hallucinations, behavioural, language and communication problems. Negative symptoms include social withdrawal, emotional flattening and reduced body language, eye contact and responsiveness. Negative symptoms are more difficult to diagnose. It requires observation of the patient over time and preferably a description of resent changes in personality by relatives.

Schizophrenia tends to develop between the ages of 16 and 30 years, and mostly persists throughout the patient's lifetime. Women tend to have a later onset than men. The illness shows a lifetime prevalence of about 1% in the general population (Mueser and McGurk). According to WHO, schizophrenia is the fifth most disabling disease worldwide. 10% of all disabled citizens in Norway have schizophrenia, and the overall cost per year for schizophrenia is estimated to 4 billion Norwegian kroner (NOK) (1995) (Johannessen). The costs for drug treatment of schizophrenia have been increasing the last years and were 370 million NOK in 2003 (Fig 1-1) (Norwegian Institute of Public Health).

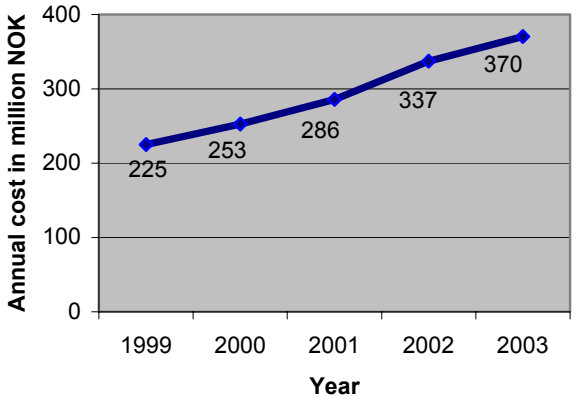

Fig 1-1: *Annual costs of antipsychotic drugs in Norway in NOK (millions)*

The human suffering associated with schizophrenia is enormous – for patients, families, and in society at large. In western countries costs are estimated to exceed 1% of the national budget. There is no other disorder with comparable costs. Schizophrenia alone costs more than all types of cancer and more than all cardiovascular diseases (Johannessen).

There are reasons to believe that not all patients with schizophrenia are given treatment in accordance with good clinical practice. The lag time from onset of manifest psychosis and to appropriate treatment is often long, in western countries up to 2-3 years (Melle et al.). Early detection and treatment of schizophrenia is an important challenge. Optimal management of the disease includes psychological, social and occupational therapies. Psychosocial intervention seeks to improve the management of the disorder (e.g. coping with symptoms, relapse prevention) and enhance functioning in areas such as independent living, relationships and work. Pharmacotherapy is the mainstay of treatment, without which most psychosocial treatment would not be possible.

1.2 Pharmacotherapy of schizophrenia

The first antipsychotics were developed more than 50 years ago. Introduction of chlorpromazine in 1952 opened possibilities to de-institutionalize psychotic patients. The antipsychotics are divided in two groups: the first-generation antipsychotics also termed typical, and the second-generation antipsychotics termed atypical. Pharmacological receptor binding profile is the main difference between the two groups. Examples of marketed antipsychotics are provided in table 1-1.

1.2.1 Typical antipsychotics

The typical antipsychotics are characterized by high *in vitro* binding affinities for the dopamine D_2 receptor. They have good antipsychotic effect, but their use is limited by common and sometimes severe side effects. This includes extrapyramidal effects like akathisia, rigidity and tardive dyskinesia, but also sedation (Freedman). There are two subgroups of typical antipsychotics; the high potency (low dosed <30 mg daily) and the low potency (high dosed >100 mg daily). The potency in binding affinities for the dopamine receptor separates the two groups.

1.2.2 Atypical antipsychotics

Clozapine was reintroduced in the late 1980s as a prototype for the atypical antipsychotics, and has led to significant advances in the pharmacological management for schizophrenia. This pharmacological progress has been attributed to 5-HT_{2A}-antagonism and a diverse binding profile on several receptors in the CNS (e.g. serotonergic, dopaminergic, histaminergic, adrenergic and cholinergic receptors). Atypical antipsychotics are also superior to typical antipsychotics in improving cognitive functioning and negative symptoms, an important factor in treatment of schizophrenia (Mori et al.). The other atypical antipsychotics have been pharmacologically modelled, to a certain extent, after clozapine; the common feature being a strong 5-HT_{2A}-antagonist. Atypical antipsychotics are better tolerated than the typical, but some of the atypical substances may still induce side effects like weight gain, sedation and metabolic complications (e.g. diabetes mellitus). It is clear that weight gain can undermine compliance, and may also lead to significant psychological distress and medical morbidity and mortality. A poor response to treatment is still considered a problem in schizophrenic patients. Loss of clinical efficacy may be caused by interindividual differences in enzyme systems and drug metabolism. In spite of increased use and introduction of new atypical antipsychotics the last years, typical antipsychotics are still commonly prescribed.

Table 1-1: *The typical and atypical antipsychotics prescribed to patients in this project:*

TYPICAL ANTIPSYCHOTICS	ATYPICAL ANTIPSYCHOTICS
Haloperidol (Haldol®)	Clozapine (Leponex®)
Zuclopenthixol (Cisordinol®)	Olanzapine (Zyprexa®)
Perphenazine (Trilafon®)	Risperidone (Risperdal®)
Flupentixol (Fluanxol®)	Quetiapine (Seroquel®)
Levomepromazine (Nozinan®)	Amisulpride (Solian®)
Thioridazine (Melleril®)	Ziprasidon (Seldox®)
Chlorprothixene (Truxal®)	

Relapse rates during two-year studies are shown to be approximately 30% during treatment with first generation antipsychotics and 80% without treatment (Marder et al.). The relapse rates are also shown to be different between first- and second-generation antipsychotics. In a one-year, multisite study by Csernansky *et al*. patients taking the second-generation drug risperidone had 25% relapse, as compared with a rate of 40% for patients taking the first-generation drug haloperidol (Csernansky, Mahmoud and Brenner).

1.2.3 Norwegian guidelines for the use of antipsychotics

Patients being hospitalized for first episode of schizophrenia should preferably be drug free for two weeks or until diagnose is established. This is to ensure an adequate observational period to establish the correct diagnose and treatment. Norwegian guidelines suggest monotherapy for the treatment of schizophrenia. Initial doses should be low, and long term dosing should be kept at a minimum level if possible. Benzodiazepines can temporarily be used to treat anxiety, restlessness and disturbed sleep. Health care personnel should be aware of side effects and adverse drug reactions (Statens Helsetilsyn.).

After the first episode of schizophrenia pharmacologic treatment should be maintained for at least two years. Patients with more than one episode of schizophrenia should receive antipsychotic treatment for at least 5 years. Patients that show relapse, suicidal attempts or violent and aggressive behaviour should receive treatment for more than five years, in most cases life-long treatment (Statens Helsetilsyn.).

1.2.4 Use of antipsychotics in Norway

Consumption of antipsychotics in defined daily doses (DDD) showed a decreasing trend during the 1990s. The patients in psychiatric hospitals received 1.49 DDD antipsychotics in 1991 and 1.22 DDD in 2000, a 19 % decrease. Since 2000 the consumption has been growing. The use of atypical drugs has shown a steady increase, from 7% in 1990 to 53% of totally prescribed antipsychotics in 2000 (Rytter and Haberg).

1.3 Metabolism of antipsychotics

Most lipophilic compounds such as psychotropics need to be metabolized prior to excretion in the urine. Lipophilic drugs are converted into more hydrophilic, usually less active or inactive metabolites. Drug metabolic reactions may be divided in two categories; phase I and phase II. Phase I involves modifications such as oxidation, dealkylation, hydrolysis and reduction. Phase II metabolism involves conjugation of glucoronide or sulphate to the parent drug or its phase I metabolites. Genetic and environmental factors heavily influence drug response. The efficacy of antipsychotic drug therapy is still difficult to predict in the individual, despite of the increasing recognition of possible pathological factors involved in schizophrenia. Genetic variability exists both at receptor, drug transporter and metabolism level. Genetic differences in the activity of metabolizing enzymes have been shown to be the most important source of variability in drug response. Phase I enzyme system cytochrome P450 (CYP) are responsible for metabolism of most of the typical and atypical antipsychotics.

1.3.1 Cytochrome P450 monooxygenase

CYP is a superfamily of phase I enzymes responsible for metabolism of many drugs and xenobiotics in the human body. They are haeme containing and derived their name from their absorption of light at 450 nm. The CYP superfamily is responsible for 70-80% of all phase I dependent metabolism, and are estimated to be involved in metabolism of more than 50% of clinically used drugs (Bertz and Granneman); (Evans and Relling). The vast majority of psychotropic drugs are metabolized by CYP, which makes CYP an important contributor to variability in psychiatric drug treatment. CYP enzymes are mainly localized in the endoplasmatic reticulum of liver cells, but also in the intestine and lungs.

More than 30 different subtypes are identified in humans, and they are categorized and named after homology of the amino acid sequence. See fig 1-2. Families share 40% homology (e.g. CYP2), subfamilies share 60% homology (e.g. CYP2D) and the subfamilies are divided into isoenzymes designated by an Arabic number (e.g. CYP2D6) (Rogers, Nafziger and Bertino, Jr.).

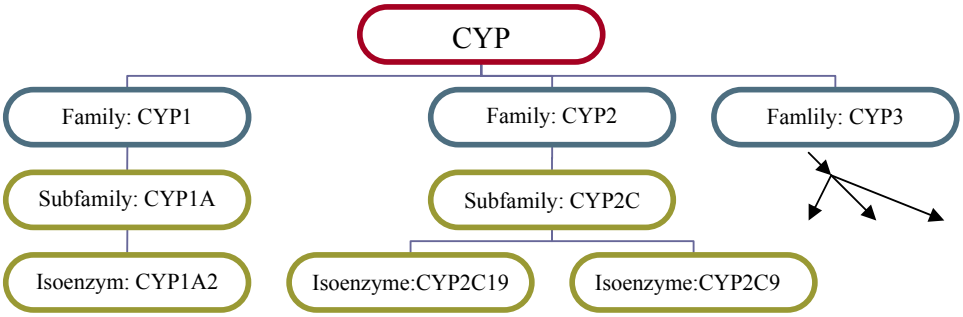

***Fig 1-2**: Grouping and nomenclature of the CYP450 superfamily*

More than 30 CYP isoenzyme systems are characterized, but only a few of them are important in the metabolism of drugs. CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4 are the far most abundant isoenzymes. Responsible for more than 90% of all CYP mediated drug metabolism (Fig 1-3) (Bertz and Granneman).

1.4 Genetic differences of CYP activity

The activity of the different CYP-enzymes shows great intra- and interindividual variation, due to both environmental and genetic factors. Genes that exist in different variants, because of evolutionary mutations, are termed polymorphic. Polymorphism refers to a genetic variant of a gene that occurs in more than 1% of a population. Genetic polymorphism is well described for isoenzymes CYP2C9, CYP2C19 and CYP2D6 (Ingelman-Sundberg).

Genetic variability will derive not only from genotype in the translated gene regions, but also from variability in gene expression and regulation. Environmental factors like cigarette smoke, certain drugs and foods can influence CYP-activity. CYP1A2 and CYP3A4 are important isoenzymes, responsible for more than 50% of CYP mediated metabolism. See fig. 1-3. None of them exhibit genetic polymorphism, but both of them are highly influenced by environmental factors.

There are different ways of obtain information about a persons CYP activity. A CYP genotyping will provide information about the subject's genetic status, valid for the rest of the life. CYP-genotyping is currently performed by polymerase chain reaction (PCR); this technique is used to synthesize large quantities of specific DNA fragments. By DNA-synthesis, subsequent specific restriction enzyme digestion and separation of the DNA fragments it is possible to analyse for mutations and variants of genes. In this project mutations of isoenzymes CYP2C9, CYP2C19 and CYP2D6 are studied.

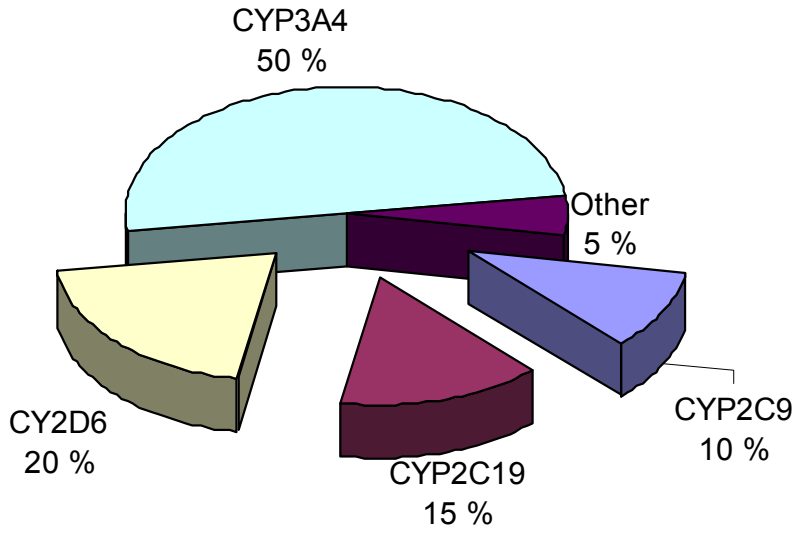

Fig 1-3: *Fractional share, in total CYP activity, of different isoenzymes.*
CYP2C9, CYP2C19 and CYP2D6 exhibit genetic polymorphism (Ingelman-Sundberg)

1.4.1 Genetic polymorphism

Mutations in the CYP genes might lead to abolished, decreased or increased activity of the different isoenzymes. Abolished enzyme activity is commonly seen where both genes has been deleted, but has also its origin in mutations causing altered splicing, stop codons, abolished transcriptional start sites and deleterious amino acid changes. Mutations in substrate recognition sites (SRS) can cause the synthesis of enzymes with altered substrate specificity. Amino acid changes might lead to changes in protein folding and thereby altered specificity of the SRS. Mutations of the different enzymes are named chronologically as they are identified, with a star (*) and an Arabic number. Reference variants (the first one detected) are termed *1, different mutations are then named *2, *3 etc. For practical reasons *1 will be used to designate all other gene variants than those analysed for in this project (wild type).

1.4.2 Polymorphic isoenzymes

CYP2C9 enzymes are responsible for 10% of total CYP mediated metabolism. Important 2C9 substrates are NSAIDS, warfarin and phenytoine. This isoenzyme is not responsible for metabolism of any antipsychotic drugs. Three mutations were screened in this project: CYP2C9 *2, *3 and *5. All of them code for lower effect, but the impact of a *2 mutation is limited.

CYP2C19 enzymes are responsible for 15% of total CYP mediated metabolism. Important 2C19 substrates are tricyclic antidepressive drugs (TCA), benzodiazepines, antiepileptics, citalopram, moclobemide and omeprazole. This isoenzyme is not responsible for metabolism of any antipsychotic drugs. Four mutations were screened in this project: CYP2C19 *2, *3, *4 and *5. *2 and *3 code for defect enzyme, *4 and *5 code for no enzyme production.

CYP2D6 enzymes are responsible for 20% of total CYP mediated metabolism. It is involved in metabolism of seven of the fifteen antipsychotic drugs marketed in Norway (haloperidol, risperidone, zuclopenthixol, levomepromazine, perphenazine, chlorprothixene, flupenthixole). Three antipsychotics (perphenazine, levomepromazine and thioridazine) are reported to act as inhibitors on CYP2D6 metabolism (Ieiri et al.). CYP2D6 are not induced by any known substance. A small group of northern European Caucasians show a high activity in the CYP2D6 activity due to duplication of the CYP2D6 gene.

1.4.3 Genotypes

Evolutionary mutations in the genes exist in a population, causing some genes to be altered or defect. The human genetic code is diploid, i.e. every person carries two sets of every gene. A person may therefore carry two, one or no functional sets of a gene. For isoenzyme CYP2D6 one of the mutations code for a duplication of the gene. In this case it is possible to carry more than two active genes coding for functional CYP2D6 enzyme, a situation only described for CYP2D6. A population can be divided into 4 different subgroups reflecting the number of functional genes coding for an isoenzyme:

No functional genes: Poor metabolizer (PM). This group is also termed homozygote positive for a non functional mutation (e.g. homozygote CYP2D6 *5 / *5).

One functional gene: Intermediate metabolizer (IM). Persons with one normal and one defect copy of a gene are referred to as IM. A mutation in one of the gene copies is not always clinically relevant. IM are also termed heterozygote positive for a given mutation (e.g. CYP2D6 *1 / *4).

Two functional genes: Extensive metabolizer (EM). This is the normal (wild type), both gene copies code for functional enzyme. (e.g. CYP2D6 *1 / *1).

More than two functional genes: Ultra rapid metabolizer (UM). This genotype exists only for the CYP2D6 isoenzyme, where the *2 mutation code for a duplication of the gene. No exact cut-off point, however, exists or has been defined for the UM-group. This is partly explained by different number of functional gene copies (up to 13 copies have been described (Dalen et al.)) and because different methods are used to measure enzyme activity.

Table 2-1 describes the mechanism of all the different CYP mutations tested in this project.

The classification based on number of active genes, is used primarily to describe CYP2D6 genotype. The system is also applicable to the other isoenzymes. PM genotype results in lack of metabolic capacity, increased exposure of parent drug or decreased exposure of active metabolites. Relevance of IM genotype will range from very low metabolic capacity, to

normal activity. UM will have extraordinary high metabolic capacity. Subjects that are PM or UM are more prone to drug levels outside the therapeutic range, loss of effect or increased risk of adverse drug reactions (ADR).

1.4.4 Ethnical differences of CYP genoptype

The occurrence of mutations in CYP-isoenzymes shows great variation between different ethnic groups. There are also differences between subpopulations within the same ethnic group (e.g. Caucasians). Frequencies of multi-duplicated CYP2D6-genes is about 1-2% in Swedish Caucasians, 3,6% in Germany, 7-10% in Spain and 10% on Sicily in Italy. In contrast the frequency is as high as 20% in Saudi Arabians and 29% in black Ethiopians (Bertilsson et al.).

1.5 Environmental changes of CYP activity

Drug metabolism may be changed by alteration of enzymatic functions by certain drugs, environmental chemicals (e.g. cigarette smoke) or foods.

1.5.1 Inhibition and induction by drugs

Drugs can act both as inhibitors or inducers of CYP activity. An inhibition might cause the patient to become a functional PM. All isoenzymes except for CYP2D6 are inducible by other drugs, causing increased CYP activity. Drug to drug interactions are a potential problem, because of inhibition and induction, when polytherapy is applied. Concomitant use of antiepileptics and antidepressants are potential causes for pharmacokinetic drug interactions in this group of patients. Concomitant use of CYP inhibitors might cause extensive metabolizers to exhibit poor metabolizer phenotype.

1.5.2 Cigarette smoking

Polycyclic aromatic hydrocarbons (PAH) from cigarette smoke are potent inducers of isoenzyme CYP1A2 (van der Steijns and van Weelden). This isoenzyme is responsible for most of the CYP450 metabolism of both clozapine and olanzapine, both commonly used in the treatment of schizophrenia. Studies indicate that approximately 70-80% of patients with schizophrenia smoke, compared to 28% of the general population (Statistics Norway).

1.6 Prediction and monitoring of drug response

Polymorphism of clinical significance for pharmacological treatment can be identified by CYP-genotyping. However enzymes like CYP1A2 and CYP3A4 does not exhibit polymorphism. They require phenotyping or systematic use of therapeutic drug monitoring to estimate enzyme activity. Phenotyping is sensible for environmental- as well as genetic factors and provides information of the patient's CYP-status at the time of phenotyping. Genotyping or phenotyping may indicate the response to a substance, and may identify possible pitfalls in pharmacological treatment. It can contribute to an optimal drug regime and confirm or support decisions based on TDM-data.

1.7 Therapeutic drug monitoring

Response to pharmacological treatment depends on a range of factors, including pharmacokinetic and pharmacodynamic differences. Serum concentrations after administration of a set dose can vary 10-20 times, in some cases up to 100 times (Bengtsson). For all the antipsychotics there are established a reference range of serum concentrations, but a therapeutic window is not defined for all atypical antipsychotics. Clinical practice is often based on subjective assessments of efficacy and side effects. Even atypical antipsychotics may produce side effects that resemble symptoms of the disease. TDM is important in establishment of new treatment, but also when changes in patient response occur. TDM might reveal zero amount of drug in the body (e.g. complete non-compliance), serum concentrations lower than expected (e.g. defects in absorption, non-compliance or increased clearance) to extremely high concentrations (e.g. overdosing, decreased clearance or drug-drug interactions). Several measures of through concentrations, over a period of time, will provide further information to guide therapy of the patient.

Basis/reasons for performing TDM in psychiatric patients:
-patients not responding to pharmacological treatment as expected
-patients in need of long term treatment
-narrow therapeutic index of drug
-subjects with deviation in pharmacokinetics or -dynamics due to age, or somatic conditions
-polypharmacy, drug-drug interactions, use of enzyme inhibitors or inducers
-prior and after changes in drug regime

1.8 Aim of study

The aim of this study was to investigate the use of psychotropics in schizophrenic patients living in nursing homes. Further, we wanted to study the impact of genetic and environmental factors on systemic serum concentrations of antipsychotics in this group of patients.

The following questions will be addressed:

What kind of pharmacological strategies are used in this population of psychiatric inpatients on long-term psychotropic treatment?
What is the frequency of poor- and ultra rapid metabolizers in this patient group compared to a Norwegian Caucasian control group?
How do CYP-mutations influence the serum concentrations in this group of patients?
How do drug-drug interactions (polypharmacy) influence the serum concentrations of antipsychotics in this group of patients?
How does cigarette smoking as well as daily consumption of cigarettes influence the serum concentrations in this group of patients?

2 METHODS

2.1 Recruiting patients

The project was initiated in 2003 by Dr. Per Håkon Eikeseth, Vor Frue Hospital. Application to the regional ethic committee was submitted by August 2003, and approved 22nd September 2003 by the Regional Ethics Committee of Health Region 1 (Ref number: 410-03139)

All patients recruited were living in nursing homes in the Oslo area. They all received long-term treatment with antipsychotic medication and were considered to be stable in respect to both their medical condition and drug regime. The patients were all previously diagnosed with severe mental illnesses.

A short interview was carried out to collect informed consent from the patients. Patient information was collected from records and medicine cards. The standard form from Department of Psychopharmacology was filled out to collect most of the patient information.

2.1.1 Collected patient information

Following patient information were collected:

-date of birth, gender

-onset and duration of psychiatric disease

-height & weight

-Body Mass Index

Defined with the formula: ((height - cm) / (weight-kg x weight-kg)).

BMI <18 is considered underweight

BMI 18-27 is normal

BMI 27-29.9 is overweight

BMI score over 30 is considered obesity.

-smoking behaviour

-information regarding use and dosage of antipsychotic- and concomitant medication

-Only drugs that were used on a regular basis were included

-Only three concomitant substances were registered, but in addition the total number of concomitant substances is registered

-duration of current antipsychotic drug regime and time since last change of dosing or antipsychotic regime (in months).
-time since administration of last dose of each antipsychotic
-blood samples for obtaining CYP-genotype and serum concentrations of antipsychotics

2.1.2 Study design

Inclusion criteria:
-ICD-10 F20.0-F39.0 (*)
-Caucasian
-Inpatient
-Treated voluntarily with antipsychotic medication
-Capable of understanding and signing the informed consent
-Stable antipsychotic drug regime, no changes of dosing or substances within the last 28 days.
* = a few of the patients had diagnoses other than schizophrenia

Exclusion criteria:
-Current drug abuse
-Current alcohol abuse
-Recent acute episode of psychiatric disease
-Somatic diseases causing altered renal or hepatic metabolic capacities.

2.2 Patient population

The population consisted of 109 inpatients from different psychiatric nursing homes around Oslo. All ICD-10 diagnoses from F20.0-F39.0 were allowed, but most of the patients (n=90) fulfilled the criteria for schizophrenia (F20.0-F20.9). Each subject signed an informed written consent in order to participate in the study.

Study nurses collected two vials of venous blood from each patient. Blood samples were collected to measure serum levels (trough) of all antipsychotics and to determine CYP-genotype. The vial of whole blood for genotyping (PCR) contained EDTA to prevent clotting. The vial for TDM was allowed to clot for 30 minutes before centrifugation and decantation of serum. Vials and form were immediately marked with analysis-number and patient initials.

2.3 Control group

To compare the frequencies of CYP-mutations in the general Caucasian Norwegian population, a control group was established. The control group consisted of 136 volunteers, recruited from health care personnel and students at the University of Oslo. All signed an informed written consent. Information from the control group only included date of birth and gender. One vial of venous blood containing EDTA was collected from each member of the control group.

2.4 Analytical methods

Analysis of antipsychotic serum levels by HPLC-MS/MS

Blood samples were analysed at the Department of Psychopharmacology, Diakonhjemmet Hospital Oslo. The separation and quantification (of all antipsychotics) was achieved using high-performance liquid chromatography (HPLC), mass spectrometry (MS). The LC-MS/MS system consisted of Waters 2695 Liquid Chromatograph (Milford, MA, USA), and mass spectrometric detection was carried out using Micromass Quattro micro ™ (Milford, MA, USA) operating in the positive electro-spray mode (AP-ESI).

All analytical methods are performed in accordance with the quality handbook of Department of Psychopharmacology, Diakonhjemmet Sykehus.

2.4.1 Sample preparation and analysis of blood samples

Blood samples were allowed to clot for 30 minutes, centrifuged and transferred to new vials as serum. The sample preparation was performed with two different methods: Liquid-liquid extraction (LLE) and protein precipitation. All antipsychotic drugs are analysed with same equipment and analytical procedures, but in three groups based on time of retention:

-Mass spectrometry run 1 (MS1) - protein precipitation: Olanzapine and chlorprothixene
-Mass spectrometry run 2 (MS2) - liquid-liquid extraction: Amisulpride, risperidone, clozapine, haloperidol, levomepromazine
-Mass spectrometry run 3 (MS3) - liquid-liquid extraction: Quetiapine, ziprasidone, chlorpromazine, zuclopenthixol, flupenthixole

2.4.2 Protein precipitation

To 500µl serum the following chemicals were added:
600µl Acetonitrile: Methanol (90:10). This solution also contained 0,5 µl promazine 1mM (internal standard). All samples were mixed 15 seconds on whirl-mixer and then cooled down for 10 minutes (-20ºC). The samples were centrifuged for 10 minutes. 200µl of the supernatant were transferred to new vials, corked with a septum and placed in an autosampler.

2.4.3 Liquid-liquid extraction

Minimum 1 ml of serum was required.
The following chemicals were added to 1 ml serum:
-120 µl 1,0M NaOH and 50 µl 20µM, promazine (internal standard).
-3 ml isopropylamine, hexan 0.1:99.9
-2 ml isopropylamine, etylacetate 0.1:99.9
All vials were extracted 10 minutes on rotating mixer at 20 RPM and then centrifuged 10 minutes at 3500 RPM. Samples were placed in -80ºC for 6-7 minutes to allow the water phase to freeze. The organic phase were transferred to new vials, heated to 37-40ºC and dried under a flow of nitrogen gas for 25 minutes. Samples were re-dissolved in mobile phase: 200µl acetonitrile, 10mM ammoniumacetate pH 4.5 (30:70). The samples were vortex mixed for 5 minutes and placed in autosampler to be analysed.

2.4.4 Quality controls

Every run was set up with the following quality controls (control samples 2 through 8 went through the same sample preparation as the patient samples):
1) Blank mobile phase
2) Drug free serum (blank)
3-6) Standards (prepared by adding 50 µl stock solution to 450µl blank serum) these four samples contain different concentrations of every antipsychotic included in the run. Results are used to obtain a standard curve.
7) Control: low range. Blank serum spiked with an exact low concentration of every antipsychotic in the run.
8) Control: high range. Blank serum spiked with an exact high concentration of every antipsychotic in the run.

2.4.5 Analysis and identification

The separation was performed on an ACE 3AQ column, C_8/C_{18} (100 x 2.1 mm, 5 μm), protected by an ACE 3AQ guard column (15 x 2.1 mm), both from TechnoLab. Time of retention and mass of the molecule ion was used for identification. Peak area was used for quantification by relating peak ratio [area drug/ area internal standard] to the peak ratio of the standard curves.

2.4.6 Precision of the analytical results

Precision was monitored as described in the quality handbook of the Department of Psychopharmacology. Coefficient of variation (CV) are recorded for all analyses on a regular basis, and denoted in percent. The CV was determined from the actual results of the high and low controls. CV are also known as the relative standard deviation and obtained by the following formula: 100 x standard deviation / mean. Values from the period when all blood samples were analysed were recorded. CV values for analytical procedures of all antipsychotics averaged 6.9% and ranged from 4.4-10.8% (n=24).

2.5 Genotyping

All genotyping were performed according to the quality handbook of the Department of Psychopharmacology. Venous blood samples were collected in vials containing ethylenediaminetetraacetic acid (EDTA) to prevent clotting. Leukocyte-DNA was isolated by solid phase extraction.

2.5.1 Extraction of leukocyte DNA

DNA was extracted with a "QIAmp - DNA Blood Mini Kit":

-Protease, 200µl whole blood and a buffer were mixed to lyse blood cells and break down proteins (e.g. enzymes and histones).

-200µl ethanol was added to modify the solubility before application of DNA to the column.

-The mixture was applied to the silica column and allowed to bind for 1 minute.

-The column was washed two times with 500µl buffer.

-Eluation of the DNA was performed with 200µl AE-buffer.

200µl whole blood yields 3-12µg DNA, consisting of fragments up to 50kb in size. Most of the DNA fragments will be approximately 20-30kb.

2.5.2 Polymerase chain reaction (PCR)

DNA from the extraction was mixed with the following:

-heat stable DNA-polymerase

-primer

-all nucleoside triphosphates (A, C, G and T)

-water, buffer and $MgCl_2$

A temperature program was run to produce a sufficient number of copies of the wanted DNA fragments. Different primers were used to produce the specific fragments. The PCR reactions were carried out on a Dyade Thermal Cycler (MJ Research, VWR international)

General procedure for the PCR reaction:

-**Denaturation**: The target DNA was heated to 95°C so that complementary strands separate.

-**Annealing**: The temperature was lowered to 54-60°C (temperatures of annealing are variable among the methods and are only indicated by this interval). Primers with a sequence complementary to the strands were allowed to bind. Because primers were added in excess, the strands mostly annealed to the primer instead of each other. Primers are constructed to prevent primer-dimerization or production of secondary structures.

-**Extension**: The temperature was increased to 72°C. The heat stable DNA polymerase extended the primers with nucleoside triphosphates and produced copies of the target sequence.

This process was repeated 21-35 times (depending on method), to produce sufficient number of copies.

2.5.3 Restriction cutting and electrophoresis of the target DNA

Specific restriction enzymes were added to the DNA mixture and allowed to work for 2 hours. Restriction enzymes cut the DNA in specific areas. Electrophoresis was used to separate the DNA fragments by size. Samples were filled in wells on an agarose / polyacrylamide gel-plate covered with TAE buffer to allow migration of DNA. A positive and a negative electrode were inserted in the gel and 75 volts were applied for 60 minutes. This caused the fragments

to move through pores in the gel, negatively charged DNA- molecules move towards the positive pole. Ethylene bromide was added to the gel to bind to the DNA-fragments and absorb ultra violet (UV)-light. Detection was performed with an UV-camera. The fragments were separated according to mass and size, and were detected by use of positive controls and standard sized fragments. A DNA-free sample in each run was used to rule out contamination by foreign DNA. A homozygote sample will usually appear as one band, reflecting that all DNA is either cut or uncut by the restriction enzyme. Heterozygote samples will appear as two bands; reflecting half of the DNA cut and half of the DNA uncut. See fig 2-1.

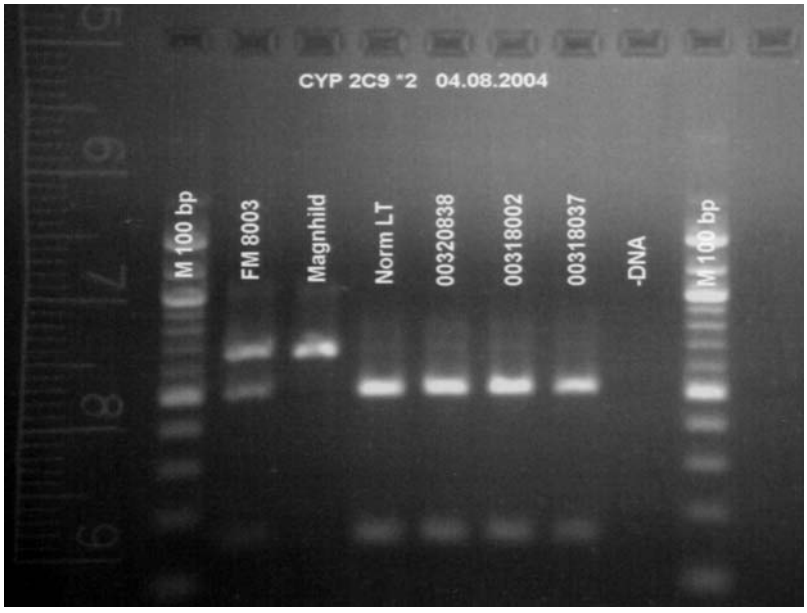

Fig 2-1: *Electrophoresis of CYP2C9*2, from the left: Fragments with standard size (M100bp), heterozygote control (two bands), homozygote control (one band, approx 700 base pairs), normal control (one band, approx 500 base pairs), 3 patients, negative control (÷DNA) and fragments with standard size (M100bp). The wild type DNA was in this case cut by restriction enzymes and therefore consisted of smaller fragments that moved further through the gel. Wells are indicated by the squares at the top of the picture.*

Table 2-1: *Mutations in CYP isoenzyme 2C and 2D6 studied*

ISOENZYME	MUT.	Mechanism of mutation	Result
CYP 2C9	*2	Exon3, 430C→T, Arg144Cys	Loss of effect
CYP 2C9	*3	1075A→C, I359L	Loss of effect
CYP 2C9	*5	1080C→G, D360E	Loss of effect
CYP 2C19	*2	Exon5, 681G→A, splicing def.	Defect enzyme
CYP 2C19	*3	Exon4, 634G→A, stop codon.	Defect enzyme
CYP 2C19	*4	GTG initiation codon, 1A→G,	No enzyme prod.
CYP 2C19	*5	R433W, 1297C→T	No enzyme prod.
CYP 2D6	*2	X N (duplication →UM)	Ultra rapid met.
CYP 2D6	*3	2549A→DEL, frameshift	Loss of effect
CYP 2D6	*4	1846G→A, splicing defect	Loss of effect
CYP 2D6	*5	CYP2D6 gene deletion (PM)	Poor metabolizer
CYP 2D6	*6	1707T→DEL, frameshift	Loss of effect
CYP 2D6	*7	2935A→C, H324P	Loss of effect
CYP 2D6	*8	1758G→T, stop codon.	Loss of effect

Mechanism of the different mutations and indication of their clinical implication are covered in table 2-1 (Bradford;Desta et al.;Lee et al.).

Long PCR (multiplex-PCR) were used to detect CYP2D6 *3, *4, *6, *7, *8. A fragment that contained the complete CYP2D6 gene were amplified and detected by gel electrophoresis. This fragment was added allele specific primers for the five mutations, went through a new round of PCR and were detected by gel electrophoresis.

CYP2C9*2, CYP2C9*3, CYP2C9*5 and CYP2C19 *2-*5 were amplified by use of allele specific primers, cutting with restriction enzymes and detection by gel-electrophoresis.

CYP2D6*2 (duplication) and CYP2D6*5 (deletion) were amplified by use of allele specific primers and detection by gel-electrophoresis. These fragments are only amplified when the mutations are present.

The analytical methods used in this project do not quantify the number of active copies in CYP2D6 gene duplication.

2.6 Entering data in SPSS

All data were entered in SPSS 11.0 for Windows (SPSS Inc., Chicago, IL, USA)

Table 2.2: Categories in SPSS. Following variables were entered in the database:

Nr.	Name	Type (unit)	Values (valid)	Additional information
1	Number	Continuous, chronological	1->	
2	Initials	String	-	
3	Institution	Categorical	1->	
4	Duration of current drug regime	Continuous, months	1->	
5	Gender	Categorical	1, 2	1=male 2=female
6	Age	Continuous, years		
7	Onset of psychiatric disease (age)	Continuous, years	1->	
8	Duration of psychiatric disease	Continuous, years		
9	Height of the patient	Continuous, centimetres	1->	
10	Weight of the patient	Continuous, kilograms	1->	
11	Body mass index	Continuous, (kg/m x m)		
12	Number of cigarettes smoked per day	Continuous, cigarettes	0->	
13	Antipsychotic medication ranked # 1	Categorical, generic name	1 to 15	
14	Daily dose of antipsychotic # 1	Continuous, milligrams		
15	CD-ratio of antipsychotic #1	Continuous, ((nmol/L)/(mg))		
16	Serum concentration antipsychotic # 1	Continuous, (nmol/L)		
17	Antipsychotic medication ranked # 2	Categorical, generic name	1 to 15	
18	Daily dose of antipsychotic # 2	Continuous, milligrams		
19	CD-ratio for antipsychotic #2	Continuous, ((nmol/L)/(mg))		
20	Serum concentration antipsychotic # 2	Continuous, (nmol/L)		

21	Antipsychotic medication ranked # 3	Categorical, generic name	1 to 15	
22	Daily dose of antipsychotic # 3	Continuous, milligrams		
23	CD-ratio of antipsychotic #3	Continuous, (nmol/L/mg)		
24	Serum concentration antipsychotic # 3	Continuous, (nmol/L)		
25	Use of any CYP2D6 substrate	Categorical	1, 2	0=no, 1=yes
26	Co medication 1	Categorical	1 to 8	
27	Co medication 2	Categorical	1 to 8	
28	Co medication 3	Categorical	1 to 8	
29	Total number of co medication drugs	Continuous		
30	Use of a CYP450 inducer	Categorical	0, 1	0=no, 1=yes
31	Use of a CYP450 inhibitor	Categorical	0, 1	0=no, 1=yes
32	Diagnosis ICD-10	Categorical	201-390	
33	CYP2C9 *2	Categorical	1, 2, 3	1=normal, 2=hetero, 3=homo
34	CYP2C9 *3	Categorical	1, 2, 3	1=normal, 2=hetero, 3=homo
35	CYP2C9 *5	Categorical	1, 2, 3	1=normal, 2=hetero, 3=homo
36	CYP2C19 *2	Categorical	1, 2, 3	1=normal, 2=hetero, 3=homo
37	CYP2C19 *3	Categorical	1, 2, 3	1=normal, 2=hetero, 3=homo
38	CYP2C19 *4	Categorical	1, 2, 3	1=normal, 2=hetero, 3=homo
39	CYP2C19 *5	Categorical	1, 2, 3	1=normal, 2=hetero, 3=homo
40	CYP2D6 *2	Categorical	1, 2, 3	1=normal, 2=hetero, 3=homo
41	CYP2D6 *3	Categorical	1, 2, 3	1=normal, 2=hetero, 3=homo
42	CYP2D6 *4	Categorical	1, 2, 3	1=normal, 2=hetero, 3=homo
43	CYP2D6 *5	Categorical	1, 2, 3	1=normal, 2=hetero, 3=homo
44	CYP2D6 *6	Categorical	1, 2, 3	1=normal, 2=hetero, 3=homo
45	CYP2D6 *7	Categorical	1, 2, 3	1=normal, 2=hetero, 3=homo
46	CYP2D6 *8	Categorical	1, 2, 3	1=normal, 2=hetero, 3=homo
47	Group	Categorical	1, 2	1= patient, 2=control
48	Does patient smoke	Categorical	0, 1	0=no, 1=yes

Table 2.3: (*) Rating of antipsychotic medication -This rating was done to simplify and guide the registration of antipsychotic medication in SPSS.

Rating	Generic name	Trade name
1	Clozapine	Leponex®
2	Olanzapine	Zyprexa®
3	Risperidone	Risperdal®
4	Quetiapine	Seroquel®
5	Ziprasidone	Zeldox®
6	Amisulpride	Solian®
7	Zuclopenthixol	Cisordinol®
8	Perphenazine	Trilafon®
9	Flupenthixole	Fluanxol®
10	Haloperidol	Haldol®
11	Thioridazine	Melleril®
12	Levomepromazine	Nozinan®
13	Chlorprothixene	Truxal®
14	Dixyracine	Esucos®

Table 2.4: Grouping of concomitant medication, denoted by ATC group (**)

Nr	ATC	
1	N03	Antiepileptic
2	N07	Antidepressive
3	N05 B/C	Anxiolytic/hypnotic/sedative
4	N04	Anticholinergic
5	N07	Other CNS
6	C	Cardiovascular drugs
7	A	GI, enzymes, antidiabetics
8	Other ATC	Other drugs

2.7 Statistical tests and methods

Parametric tests assume that the observations in each group are equally distributed and standard deviations are similar in the two groups. If this is not the situation a non-parametric method should be applied instead. To test differences in genotype between patient- and control group the chi-square test was used. Student t-test, a parametric method used to compare two independent groups of data was used when applicable. Statistical significance was considered a two-tailed $p<0.05$. Correlation is a connection between two continuous variables, and is defined by a coefficient ranging from –1 to 1. Pearson's correlation coefficient was applied because data was evenly distributed (parametric test). Spearman correlation (non-parametric) was used solely to control the results. The Bonferoni test was not applied due to the explorative and descriptive nature of this project.

2.8 Ratios

Concentration to dose ratio (CD-ratio) was obtained by dividing serum concentrations (nmol/L) with daily antipsychotic dose (mg). This parameter could indicate deviation in clearance or bioavailability. Relative CD ratio was obtained by dividing each patient's CD ratio with the mean CD ratio of this antipsychotic. This was done to compare CD ratios for different antipsychotic substances. Some of the patients were treated with depot injections. Depot doses are multiplied with a bioequivalence (bioavailability) factor of 2, so that distribution routes can be compared. The method assumes a bioavailability of 50% per oral, and 100% with parenteral administration. This is a conventional method used in psychiatry; however this is not pharmacologically correct.

2.9 Registration of concomitant medication

In the registration of concomitant medication, only three different substances were coded. All concomitant medication was investigated to discover potential drug to drug interactions. In addition to this information, the total number of concomitant drugs was recorded.

2.10 Therapeutic range, standard dosing and defined daily doses (DDD)

Therapeutic reference ranges were obtained from the literature, or from TDM of antipsychotics, but also from PK studies. Standard dosing was obtained from product résumés and PK studies. DDD were obtained from the WHO Collaborating Centre for Drug Statistics, which publish DDD for all available ATC numbers. (WHO) (Baumann et al.).

3 RESULTS

3.1 Patient age, onset and duration of illness

109 patients (73 men and 36 women) out of totally 112 screened met the inclusion criteria of the study. The patient age ranged from 28 to 86 years with a mean age of 55 years (SD 11 years). The onset of the psychiatric disease ranged from 9 to 60 years with a mean age of 24 years. The duration of the disease ranged from 3 to 60 years, mean 31 years (SD 13). The duration of the patient's current drug regime ranged from 1 to 372 months with an average of 25 months (SD 42 months). Information about changes in drug regime was obtained from patient records. It states the time from last change of dose or substance in the patient's antipsychotic drug regime.

3.2 Diagnoses

90 out of 109 patients in this study were diagnosed with schizophrenia (F20.0-F20.9). The most frequent diagnoses were paranoid- (61 patients) or hebephrenic schizophrenia (24 patients). 5 patients were diagnosed with other types of schizophrenia, 7 patients were schizoaffective and 5 patients were bipolar. See also table 3-1. The rest of the patients had different diagnoses characterized with schizophrenic or psychotic symptoms.

Table 3-1: *Diagnoses according to ICD 10*

Number of patients	ICD10 Code	Diagnosis
61	F20.0	Paranoid schizophrenia
24	F20.1	Hebephrenic schizophrenia
1	F20.2	Catatonic schizophrenia
1	F20.3	Undifferentiated schizophrenia
1	F20.5	Residual schizophrenia
2	F20.9	Unspecified schizophrenia
7	F22.0	Paranoid psychosis
4	F25.0	Schizoaffective, manic type
3	F25.2	Schizoaffective, mixed type
5	F31.6	Bipolar affective

3.3 Pharmacotherapy of the patient group

The patient group received treatment with an average of 1.5 different antipsychotics (54 patients received one, 43 patients two and 12 patients received three different antipsychotics). See table 3-3. The patients used an average of 2.2 DDD (SD 1.5) of antipsychotic medication. Primary antipsychotics were dosed an average of 1.6 DDD (SD 0.8) in our patient group. Secondary antipsychotics were dosed an average of 1.0 DDD (SD 1.3) and tertiary antipsychotics were dosed an average of 0.3 DDD (SD 0.2).

Blood serum levels were tested for 171 antipsychotics. The most commonly used drug was olanzapine (43 patients), clozapine (33 patients), levomepromazine (23 patients), risperidone (16 patients), perfenazine (15 patients), zuclopenthixol (15 patients) and haloperidol (10 patients). See table 3-3.

***Table 3-2**: Patients treated with different antipsychotics as primary, secondary and tertiary substance.*

Primary antipsychotic	n	Secondary antipsychotics	n	Tertiary antipsychotics	N
Clozapine	32	olanzapine	1	perfenazine	1
Olanzapine	42	risperidone	4	levomepromazine	9
Risperidone	12	quetiapine	2		
Quetiapine	2	ziprasidone	1		
zuclopenthixol	6	amisulprid	2		
perphenazine	5	zuclopenthixol	9		
flupenthixole	2	perfenazine	9		
Haloperidol	4	flupenthixole	2		
thioridazine	2	haloperidol	6		
		thioridazine	1		
		levomepromazine	14		
		chlorprotixene	3		
		dixyrazine	1		

Table 3-3: *Generic and brand name of antipsychotics, number of patient per substance, DDD (defined by WHO) and CYP-isoenzyme involved (WHO), (Dahl).*

Substance	Trade name	n-patients	DDD	CYP-ENZYME
Olanzapine	Zyprexa ®	43	10mg	CYP1A2
Clozapine	Leponex ®	33	300mg	CYP1A2
Levomepromazine	Nozinan ®	23	300mg	CYP2D6
Risperidone	Risperdal ®	16	5mg	CYP2D6
Perfenazine	Trilafon ®	15	30mg	CYP2D6
Zuklopenthixol	Cisordinol ®	15	300mg	CYP2D6
Haloperidol	Haldol ®	10	8mg	CYP2D6
Quetiapine	Seroquel ®	4	400mg	CYP3A4
Flupenthixole	Fluanxol ®	4	6mg	CYP2D6
Chlorprotixene	Truxal ®	3	30mg	CYP2D6
Thioridazine	Melleril ®	3	300mg	CYP2D6
Dixyrazine	Esucos ®	1	50mg	NA
Ziprazidone	Zeldox ®	1	80mg	NA (Renal)

3.4 Serum concentrations and dosing of antipsychotics

Data were only included in this section for antipsychotics used by more than 10 patients. For additional information, see table 3-5. Quite a few patients had serum concentrations below or above reference range, 18% and 25% respectively. Most of the cases of serum concentrations below reference range (n=30) are caused by levomepromazine (n=22), the rest of the cases involve haloperidol (n=4); perphenazine (n=2); quetiapine (n=1) and risperidone (n=1). See table 3-4.

***Table 3-4**: The number of patients that showed serum concentrations below, within or above the reference range*

Ref. range	ANTIPSYCHOTIC#1	ANTIPSYCHOTIC#2	ANTIPSYCHOTIC#3	N	%
BELOW	3	19	8	30	18%
NORMAL	70	22	1	93	57%
ABOVE	30	10	1	41	25%
TOTAL	105	50	11	164	

3.4.1 Olanzapine

43 patients received treatment with olanzapine and valid serum concentrations were collected from 42 of these patients. The average serum concentration was 157 nmol/L (SD 133), while the reference range are 30-200 nmol/L. The doses ranged from 5-50 mg daily, with an average of 20 mg (SD 9). Recommended daily dose is 10-25 mg daily. Olanzapine were used as monotherapy in 16 patients, together with typical antipsychotics in 23 patients and in combination with atypical antipsychotics in 4 patients. Dose-response relationship shows high intra individual differences of a given dose.

3.4.2 Clozapine

33 patients received treatment with clozapine. The average serum concentration was 1508 nmol/L (SD 742), while the reference range are 200-2000 nmol/L. Average daily dosing was 480 mg (SD 157), while recommended dosing of clozapine range from 50-900 mg. Clozapine were used as monotherapy in 19 patients, in combination with typical antipsychotics in 8 patients and in combination with atypical antipsychotics in 6 patients. A dose-response relationship shows high intra individual differences of a given dose.

3.4.3 Risperidone

16 patients received treatment with risperidone. The average serum concentration was 106 nmol/L (SD 62), while the reference range are 30-120 nmol/L. Average daily dosing was 5 mg (SD 3), while the recommended dosing range from 2-12 mg. Risperidone were used as monotherapy in 7 patients, in combination with a typical antipsychotics in 5 patients and in combination with a typical antipsychotics in 4 patients.

3.4.4 Perphenazine

15 patients received treatment with perphenazine. The average serum concentration was 11 nmol/L (SD 16), while the reference range are 2-6 nmol/L. Average daily dosing was 22 mg (SD 20), while recommended dosing range from 8-64 mg. Perphenazine were used as monotherapy in 2 patients, in combination with typical antipsychotics in 3 patients and together with atypical antipsychotics in 10 patients.

3.4.5 Haloperidol

10 patients received treatment with haloperidol. The average concentration was 12 nmol/L (SD 9), while the reference range are 7-30 nmol/L. Average daily dosing was 10 mg (SD 12), while recommended dosing range from 2-20 mg. Haloperidol were used in monotherapy in 3 patients, together with a typical antipsychotics in 1 patient and in combination with atypical antipsychotics in 6 patients. Four patients showed serum concentrations below reference range.

3.4.6 Zuclopenthixol

15 patients received treatment with zuclopenthixol. The average serum concentration was 43 nmol/L (SD 41), while the reference range are 7-30 nmol/L. Average daily dosing was 37 mg (SD 33), while recommended dosing range from 10-60 mg. Zuclopenthixol were used in monotherapy in 4 patients, together with typical antipsychotics in 2 patients and in combination with atypical antipsychotics in 9 patients.

3.4.7 Levomepromazine

23 patients received treatment with levomepromazine. The average concentration was 79 nmol/L (SD 55), while the reference range are 150-800 nmol/L. Average daily dosing was 64 mg (SD 104), while recommended dosing range from 10-600 mg. Levomepromazine were not used in monotherapy, in combination with typical antipsychotics in 4 patients and in combination with atypical antipsychotics in 19 patients. 22 out of 23 patients treated with levomepromazine showed serum concentrations below reference range.

Table 3-5: *Results from patient group; data are only included for antipsychotics used in more than five patients. Average values and standard deviations are included for BMI, number of daily cigarettes, age of onset psychiatric disease, serum concentrations, daily dosing and concentration to dose (CD) ratio. Reference values for serum concentrations and daily dosing are obtained from literature.*

Generic name	Brand name	Patients	BMI	n cigarettes	Age of onset
clozapine	Leponex®	33	27.6 SD 4.2	15.5 SD 9.7	22.3 SD 6
olanzapine	Zyprexa®	43	26.7 SD 5.3	13.8 SD 10.7	23.5 SD 8.2
risperidone	Risperdal®	16	25.2 SD 4.6	13 SD10.8	22.8 SD 8.7
quetiapine	Seroquel®	4	27.0 SD 3.0	7.0 SD 8.7	20.7 SD 2.9
ziprasidone	Zeldox®	1	(27.5)	(12)	(27)
Amisulprid	Solian®	2	(27.4)	(10)	(22)
zuclopenthixol	Cisordinol®	15	24.5 SD 2.9	12 SD 10.6	22 SD 6.6
perphenazine	Trilafon®	15	27.0 SD 4.0	17 SD 16.0	22.4 SD 5.6
flupenthixole	Fluanxol®	4	24.9 SD 5.0	17 SD17.4	31 SD 19.5
Haloperidol	Haldol®	10	27.6 SD 5.5	14.7 SD10.6	22.6 SD11.5
thioridazine	Melleril®	3	-	-	-
levomepromazin	Nozinan®	23	25.7 SD 4.7	17 SD 10	21.3 SD 6.6
chlorprotixene	Truxal®	3	-	-	-
dixyracine	Esucos®	1	-	-	-
		176			

Generic name	Serum concentr. (nmol/L)	Reference range (nmol/L)	Average dosing (mg)	Reference dosing	Average CD-ratio
Clozapine	1508 SD 742	200-2000	483 mg SD 157	50-900mg	3.4
Olanzapine	158 SD 132	30-200	20.4 mg SD 8.6	10-25mg	8.1
Risperidone	106 SD 62	30-120	5.0 mg SD 2.7	2-12	21.4
Quetiapine	206 SD 140	100-800	775 mg SD 206	150-750mg	-
Ziprasidon	(511)	30-200	(160 mg)	40-160mg	-
Amisulpride	(3380)	400-1500	(800 mg)	100-900mg	-
Zuclopenthixol	43.2 SD 40.5	7-30	37.0 mg SD 32.5	10-60mg	1.3
Perphenazin	10.6 SD 16.3	2-6	21.8 mg SD 19.8	8-64 mg	0.4
Flupenthixol	13.1 SD 8.0	2-8	23.0 mg SD11.5	2-12 mg	-
Haloperidol	11.8 SD 9.2	7-30	10.2 mg SD 11.8	2-20 mg	2.0
Thioridazine	-	-	-	-	-
Levomepromazin	78.6 SD 55	150-800	64 mg SD 104.5	10-600mg	0.8
Chlorprotixene	-	100-800	-	100-200mg	-
Dixyracine	-	-	-	-	-

3.5 Smoking habits

Eighty-two out of 109 patients (75%) smoked cigarettes, while 27 patients (25%) were non-smokers. The average number of daily cigarettes among smokers was 18 (SD 9). The number of cigarettes per day ranged from 2 to 50. According to a survey from 2002, 28% of the Norwegian population aged 18 or older smoked tobacco (Statistics Norway). Smoking habits are displayed in fig. 3-1.

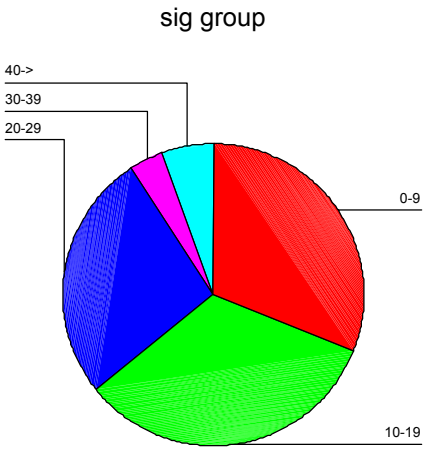

Fig 3-1: *Number of daily cigarettes*

3.5.1 Weight and BMI

Height and weight were collected from 105 out of 109 patients. BMI values ranged from 18.4 to 41.1. Average BMI was 27 (SD 5). Women had an average BMI of 29 and men a BMI of 26. Fifty-five percent of the patients had BMI values within the normal range, while 17% were considered overweight and 26% were considered obese. In the general Norwegian population aged 45-66 years 10% are considered obese. (Statistics Norway) Weight gain is one of the most important side effects of treatment with atypical antipsychotics. The average BMI of the patients that received atypical antipsychotics was not different from the patients treated with typical antipsychotics (26.8 SD=4.8 and 27.0 SD=5). Among atypical antipsychotics, only three drugs were used in more than five patients and therefore included in this section.

There were no significant differences in BMI between these three drugs, but risperidone patients seem to have lower BMI values. Average BMI for clozapine patients was 27.6 (SD=4.2), olanzapine 26.7 (SD=5.3) and risperidone 25.2 (SD=4.6). See fig 3-2.

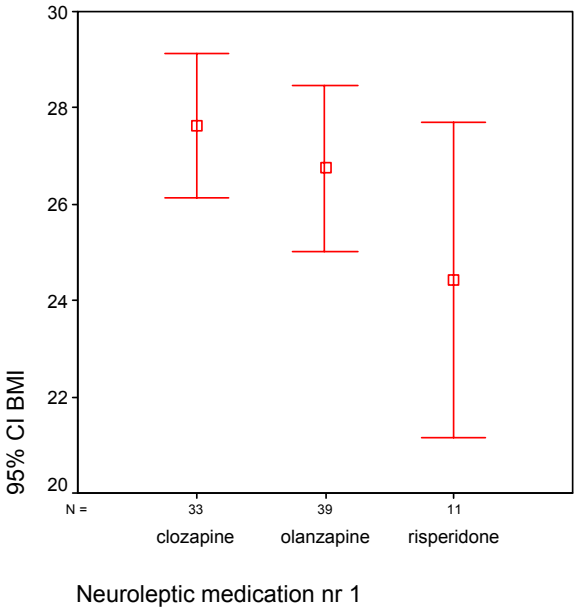

Fig 3-2: *Confidence interval (95%): BMI values of patients treated with different atypical antipsychotics*

3.5.2 Concomitant medication

Only three concomitant drugs were recorded for each patient, in addition the total number of concomitant drugs was recorded. Ninety-one out of 109 patients (83%) received at least one concomitant drug, other than antipsychotics. The average number of concomitant drugs was 2.7 (SD 2.2). One patient received 10 drugs in addition to two different antipsychotics. Anatomic therapeutic chemical (ATC) class N was the most frequently prescribed.

-ATC class N includes all central nervous system (CNS) drugs:

-ATC N03: antiepileptics were prescribed to 58 patients.

-ATC N06: psychoanaleptics were prescribed to 40 patients.

-ATCN05: hypnotics/anxiolytics/sedatives were prescribed to 39 patients.

-ATC class C, cardiovascular drugs, were prescribed to 21 patients.

-ATC C10 Serum lipid lowering agents were prescribed to 5 patients

-ATC group A (e.g. GI-tractus and diabetes, endocrinology) was prescribed to 16 patients.

-Other ATC groups were represented in 25 patients.

For additional information see table 3-6.

Table 3-6: *Concomitant medication, classified after ATC groups.*

ATC group:	Comed. # 1	Comed. # 2	Comed. # 3	Sum
N03 Antiepileptics	49	8	1	58
N06 Psychoanaleptics	14	21	5	40
N05 B/C Hypn./anxiol./sedatives	9	13	17	39
N04 Antiparkinson anticholinergics	7	5	4	16
C Cardiovascular drugs	4	12	5	21
A GI-tractus, diabetes, endocrinology	3	7	6	16
Other ATC	5	7	13	25
Sum	91	73	51	215

3.6 Impact of CYP2D6 genotype on serum concentrations

3.6.1 Ultra rapid metabolizers - CYP2D6 *2 Gene duplication

Four patients were positive for CYP2D6 gene duplication and are briefly discussed in this section:

Patient 1:

60-year-old male treated with 18 mg zuclopenthixol (0.6 DDD) in combination with olanzapine. Serum concentration of zuclopenthixol was 19 nmol/L, while the reference range is 7-30 nmol/L. The patient has no other CYP-mutations and did not receive CYP inducers or inhibitors. However the impact of gene duplication appears to be minor in this patient.

Patient 2:

60-year-old female, treated with 50 mg chlorprothixene (1.7 DDD) in combination with clozapine. Serum concentration of chlorprothixene was 20 nmol/L, while the reference range is 100-800 nmol/L. The patient has no other CYP-mutations and did not receive CYP-inducers or inhibitors. A serum concentration far below the therapeutic range and normal dosing indicates a high CYP2D6 clearance in this patient. The patient received this treatment the last 5 years prior to testing.

Patient 3:

57-year-old male, treated with 4 mg haloperidol (0.5 DDD) in combination with olanzapine. Serum concentration of haloperidol was 15 nmol/L, while the therapeutic range is 7-30 nmol/L. The patient has no other CYP-mutations and did not receive CYP inducers or inhibitors. Impact of gene duplication appears to be minor in this patient.

Patient 4:

60-year-old female treated with olanzapine and amisulpride, none of these antipsychotics are mainly metabolized by CYP2D6.

3.6.2 CYP2D6 poor metabolizers

Five poor metabolizers were found in the group: Two CYP2D6 *4 / *5 compound poor metabolizers and three CYP2D6 *4 / *4 poor metabolizers:

Patient 1:

70-year-old female treated with risperidone 5 mg (1 DDD). The serum concentration was 261 nmol/L, while the reference range is 30-120 nmol/L. Average serum concentration for the 15 other patients in the group was 106 nmol/L (SD 62), denoting that this patient has a serum concentration 2.4 times the standard deviations and a relative exposure of 2.5 times higher than the rest of the group. CD ratio for this patient was 58, while the mean CD ratio of the group was 21.4. Relative CD ratio for risperidone was 2.7.

Patient 2:

49-year-old male treated with risperidone 1 mg (0.2 DDD) in combination with clozapine. Serum concentration of risperidone was 51 nmol/L, while the therapeutic range is 30-120 nmol/L. The CD ratio of risperidone was 51 and the average CD ratio was 21.4 in the group. A high CD ratio in this patient denotes a low CYP2D6 clearance. The patient did not receive CYP inhibitors or inducers and dosing appears to be well titrated in this patient. Relative CD ratio for risperidone was 2.4.

Patient 3:

67-year-old male treated with flupenthixole 20 mg (3.3 DDD) and levomepromazine 25 mg (0.08 DDD) in combination with olanzapine. Serum concentrations were 34 nmol/L for flupenthixole (reference range: 2-8 nmol/L) and 23 nmol/L for levomepromazine (reference range: 10-600 nmol/L). No CD ratio was obtained for flupenthixole (N=4), but the CD ratio for levomepromazine was 0.92 with an average in the group of 0.83. Relative CD ratio for levomepromazine was 1.1.

Patient 4:

62-year-old male treated with zuclopenthixol 49 mg (1.7 DDD). The serum concentration was 45 nmol/L, while the therapeutic range is 7-30 nmol/L. The CD ratio was 0.92, with an average in the group of 0.32. Relative CD ratio for zuclopenthixol was 0.7.

Patient 5: Was not treated with CYP2D6 substrate.

3.6.3 Intermediate metabolizers

CYP2D6 *1 / *3

Only one patient was heterozygote positive CYP2D6 *3. This patient was not treated with CYP2D6 substrates.

CYP2D6 *1 / *4

20 of the 30 patients with a heterozygote CYP2D6*4 mutation received treatment with a CYP2D6 substrate. Relative CD ratio for the CYP2D6 substrates was 1.28 (SD=1.04). The CD-ratio denoted slightly decreased clearance but the standard deviation indicates a high degree of variation.

CYP2D6 *1 / *5

5 patients were positive for the CYP2D6 *1 / *5 mutation (deletion of the enzyme). Four of these patients were treated with CYP2D6 substrates and are briefly described in this section:

Patient 1:

54-year-old male treated with risperidone 5 mg (1 DDD). The serum concentration was 158 nmol/L, while the reference range is 30-120 nmol/L. The CD ratio of this patient was 31.6, the average in the risperidone group was 21.44. This indicates decreased risperidone clearance in this patient. Relative CD ratio: 1.47.

Patient 2:

55-year-old male treated with and zuclopenthixol 50 mg (1.7 DDD), in combination with olanzapine. Serum concentration of zuclopenthixol was 43 nmol/L, while the reference range is 7-30 nmol/L. The CD ratio was 0.86, while average in the zuclopenthixol group was 1.32. Zuclopenthixol clearance in this patient is higher than average, indicating no clinical relevance of CYP2D6 gene deletion. Relative CD ratio: 0.65.

Patient 3:

57-year-old female treated with levomepromazine 50 mg (0,17DDD) in combination with olanzapine 15 mg. Serum concentration of levomepromazine was 61 nmol/L, while the reference range is 150-800 nmol/L. The CD ratio of this patient was 1.22 compared to 0.83 for the rest of the group. A higher CD ratio than average indicates decreased clearance for levomepromazine. Relative CD ratio: 1.47.

Patient 4:

40-year-old female treated with olanzapine 35 mg and perfenazine 5 mg (0.17DDD). Serum concentration of perfenazine was 3.1 nmol/L (Ref: 2-6 nmol/L). The CD ratio of this patient was 0.60 compared to 0.39 for the rest of the group. Perfenazine clearance was higher than average for the group. Relative CD ratio: 1.54.

CYP2D6 *1 / *6

Two patients were positive for a heterozygote CYP2D6 *6, but none of them were treated with antipsychotics metabolized by CYP2D6.

3.7 Genotype of patients compared with control group

The patient group (n=109) consisted of 73 men and 36 women with a mean age of 55 years. The control group (n=136) consisted of 51 men and 85 women with a mean age of 32 years.

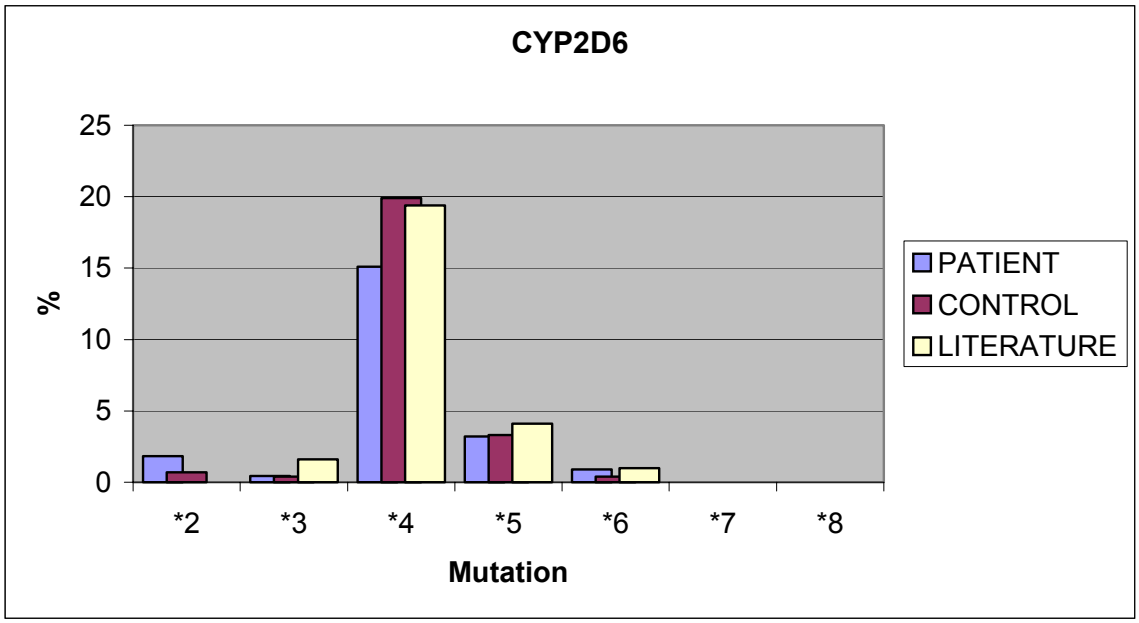

Fig 3-3: *Allele frequencies CYP2D6: patients, controls and Caucasian groups (literature)*

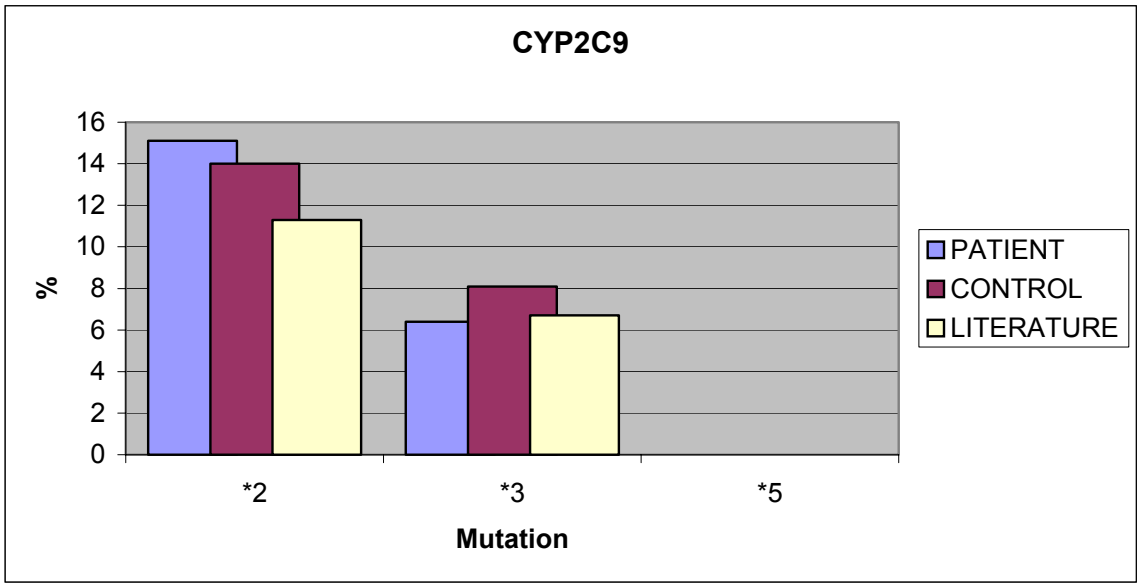

Fig 3-4: *Allele frequencies CYP2C9: patients, controls and Caucasian groups (literature)*

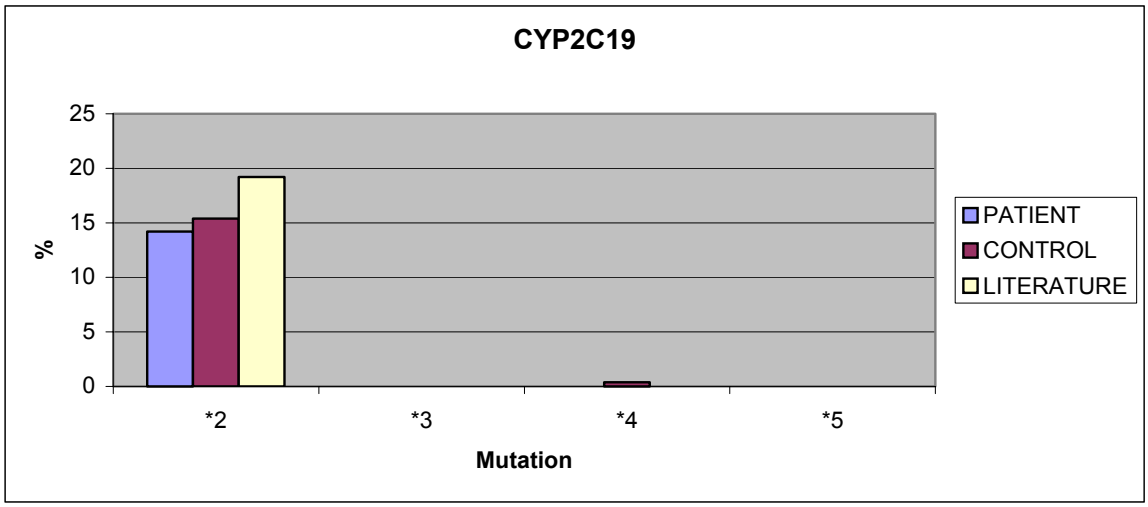

Fig 3-5: *Allele frequencies CYP2C19: patients, controls and Caucasian groups (literature)*

Table 3-7: *Allele frequencies for patient group, control group and Caucasian literature values.*

CYP2C9	PATIENT	CONTROL	LITERATURE	REF
*2	15.1	14	11.3	1
*3	6.4	8.1	6.7	1
*5	0	0	-	-
CYP2C19	PATIENT	CONTROL	LITERATURE	
*2	14.2	15.4	19.2	2
*3	0	0	-	-
*4	0	0.4	-	-
*5	0	0	-	-
CYP2D6	PATIENT	CONTROL	LITERATURE	
*2	1.83	0.7		
*3	0.45	0.4	1.6	3
*4	15.1	19.9	19.4	3
*5	3.2	3.3	4.1	3
*6	0.9	0.4	1	3
*7	0	0	-	-
*8	0	0	-	-

1. Lee CR*, et al. Pharmacogenetics* 2002; 12**:** 251-63. *(CYP2C9)*
2. Desta Z*, et al. Clin Pharmacokinet* 2002; 41**:** 913-58. *(CYP2C19)*
3. Bradford LD. *Pharmacogenomics* 2002; 3**:** 229-43. *(CYP2D6)*

***Table 3-8**: CYP2D6 genotype in patient group (n=109), control group (n=136) and total number for all genotyped subjects (n=245). No subjects were positive for CYP2D6 *7 or CYP2D6 *8 genotype and are therefore not included*

	PATIENT		CONTROL		TOTAL	
Genotype	N	Fraction	N	Fraction	N	Fraction
Poor metabolizers:						
CYP2D6 *4 / *4	3	2.8%	3	2.2%	6	2.4%
CYP2D6 *4 / *5	2	1.8%	1	0.7%	3	1.2%
Total PM CYP2D6	5	4.6%	4	2.9%	9	3.6%
Intermediate metabolizers:						
CYP2D6 *1 / *3	1	0.9%	1	0.7%	2	0.8%
CYP2D6 *1 / *4	25	22.9%	47	34.6%	72	29.4%
CYP2D6 *1 / *5	5	4.6%	8	5.9%	13	5.3%
CYP2D6 *1 / *6	2	1.8%	1	0.7%	3	1.2%
Total IM CYP2D6	33	30.3%	57	42.0%	90	36.7%
Ultra rapid metabolizers:						
CYP2D6 *2	4	3.7%	2	1.5%	6	2.4%
Extensive metabolizers:						
CYP2D6 *1 / *1	67	61.4%	73	53.7	140	57.1%
Total	109	100%	136	100%	245	100%

***Table 3-9**: CYP2C19 genotype in patient group (n=109), control group (n=136) and total number for all genotyped subjects (n=245). No subjects were positive for CYP2C19 *3 or CYP2C19 *5 genotype and are therefore not included.*

	PATIENT		CONTROL		TOTAL	
Genotype	N	Fraction	N	Fraction	N	Fraction
Poor metabolizers:						
CYP2C19 *2 / *2	2	1.8%	1	0.7%	3	1.2%
Intermediate metabolizers:						
CYP2C19 *1 / *2	27	24.8%	40	29.4%	67	27.4%
CYP2C19 *1 / *4	0	0%	1	0.7%	1	0.4%
Extensive metabolizers:						
CYP2C19 *1 / *1	80	73.4%	94	69.1%	174	71%
Total	109	100%	136	100%	245	100%

***Table 3-10**: CYP2C9 genotype in patient group (n=109), control group (n=136) and total number for all genotyped subjects (n=245). No subjects were positive for CYP2C9 *5 genotype and are therefore not included.*

	PATIENT		CONTROL		TOTAL	
Genotype	N	Fraction	N	Fraction	N	Fraction
CYP2C9 *2 / *2	0	0%	5	3.5%	5	2.0%
CYP2C9 *3 / *3	0	0%	1	0.7%	1	0.4%
CYP2C9 *1 / *2	33	30.3%	28	20.6%	61	24.9%
CYP2C9 *1 / *3	14	12.8%	20	14.7%	174	71%
Total	109	100%	136	100%	245	100%

***Table 3-11**: Allele frequencies in patient- and control group compared with the chi-square test. Allele frequencies = (number of mutations) / (number of subjects * 2). Because of the diploid human genome every person may have two mutations. Heterozygote genotype counts as one and homozygote mutations counts as two mutations.*

Mutation	Allele frequency patients	Allele frequency controls	Fishers exact test Sigma 2-sided	Significant (p=0,5)
CYP2C9 *2	15.1%	14%	0.67	NS
CYP2C9 *3	6.4%	8.1%	0.59	NS
CYP2C9 *5	-	-	-	-
CYP2C19 *2	14.2%	15.4%	0.78	NS
CYP2C19 *3	-	-	-	-
CYP2C19 *4	0%	0.4%	-	-
CYP2C19 *5	-	-	-	-
CYP2D6 *2	1.83%	0.7%	0.41	NS
CYP2D6 *3	0.45%	0.4%	-	-
CYP2D6 *4	15.1%	19.9%	0.14	NS
CYP2D6 *5	3.2%	3.3%	-	-
CYP2D6 *6	0.9%	0.4%	0.59	NS
CYP2D6 *7	-	-	-	-
CYP2D6 *8	-	-	-	-

3.8 Impact of environmental factors on the serum concentrations of antipsychotics

3.8.1 Use and impact of CYP inducers

Five out of 109 patients (4.6%) received treatment with CYP inducers. All five patients used carbamazepine in doses ranging from 400-1300 mg daily (mean 780 mg daily).
Two of these patients received only CYP2D6 substrates, not relevant here because this enzyme is not prone to induction. One patient received only a CYP3A4 substrate and the two last patients receive both a CYP1A2 and CYP2D6 substrate. The three patients treated with CYP1A2 and CYP3A4 substrates are briefly described in this section:

Patient 1:
60-year-old male, treated with quetiapine 600 mg (1.5 DDD) and carbamazepine 600 mg. Serum concentration of quetiapine was 31 nmol/L, while the reference range is 100-800 nmol/L. Subtherapeutic serum concentration indicates poor response of current quetiapine treatment. Mean CD ratio for the quetiapine group was 0.28, but this value was based on only four patients. The CD ratio of this patient was 0.05, indicating more than a fivefold increase in clearance compared with average quetiapine group

Patient 2:
50-year-old male, treated with haloperidol, levomepromazine, olanzapine 20 mg (2 DDD) and carbamazepine 400 mg. Serum concentration of olanzapine was 87 nmol/L, while the reference range is 30-200 nmol/L. The CD ratio for olanzapine was 4.35 while the mean CD ratio for the group was 8.1. A low CD ratio indicates high clearance. The patient smokes 20 cigarettes per day.

Patient 3:
48-year-old male, treated with olanzapine 35 mg (3.5 DDD) and carbamazepine 1300 mg. Serum concentration of olanzapine was 142 nmol/L, while the reference range is 30-200 nmol/L. The CD ratio was 4.06 while mean in the group was 8.1. The patient does not smoke, and the inducer effect was probably related to carbamazepine.

3.8.2 Use and impact of CYP inhibitors

No concomitant use of relevant inhibitors for isoenzyme CYP1A2, CYP2C9, CYP2C19 or CYP3A4 was registered in the patient group. Inhibitors of these enzymes include: antifungal agents, antibiotics, calcium-channel blockers, and antiviral agents. Several antidepressive agents (e.g. selective serotonin reuptake inhibitors (SSRI) fluvoxamine, fluoxetine and paroxetine) are characterized as potent inhibitors of CYP 2D6. Some of the patients used antidepressants, but none of the substances used were CYP inhibitors.

30 out of 109 patients (27.5%) used a CYP inhibitor. Levomepromazine, perphenazine and bupropione are the only CYP inhibitors used in this group. Twenty-one of these patients used both a CYP 2D6 substrate and a CYP inhibitor. None of the patients used an SSRI described as an inhibitor. One patient used bupropione (Zyban®) for smoking cessation, bupropione being a potent inhibitor of CYP2D6.

Twelve of the patients received a CYP inhibitor in combination with risperidone, zuclopenthixol or haloperidol. Average relative CD ratio in these 12 patients was 0.93 (SD 0.39), indicating normal clearance. This does not support that levomepromazine, perphenazine and bupropione are considered potent CYP-inhibitors. The inhibitors were dosed an average of 0.4 DDD, this might explain the lack of influence on clearance. Some of the patients showed high ratios, but this was also associated with intermediate metabolizer genotype. The number of patients was too low to conclude if there are differences in the potency of the different inhibitors.

3.8.3 Impact of smoking on antipsychotics metabolized by CYP1A2

The CD ratio in the clozapine group ($p=0.001$), and in the olanzapine group ($p=0.001$), and the relative CD ratio for all CYP1A2 users ($p<0.001$) were significantly lower in smokers compared to non-smokers. The non-smokers in the clozapine group had 2.1 times higher CD ratios compared with smokers. The non-smokers in the olanzapine group had 1.9 times higher CD ratios compared to smokers. See also fig 3-6, 3-7, 3-8 and 3-9.

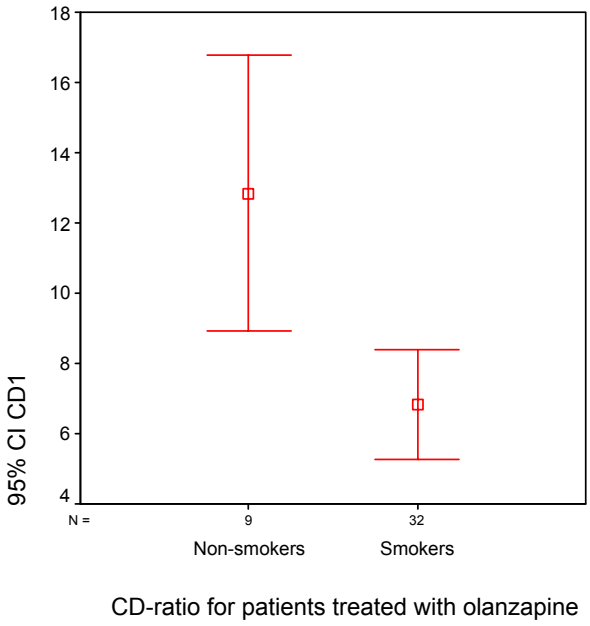

***Fig 3-6**: 95% CI for CD ratios in olanzapine smokers and non-smokers*

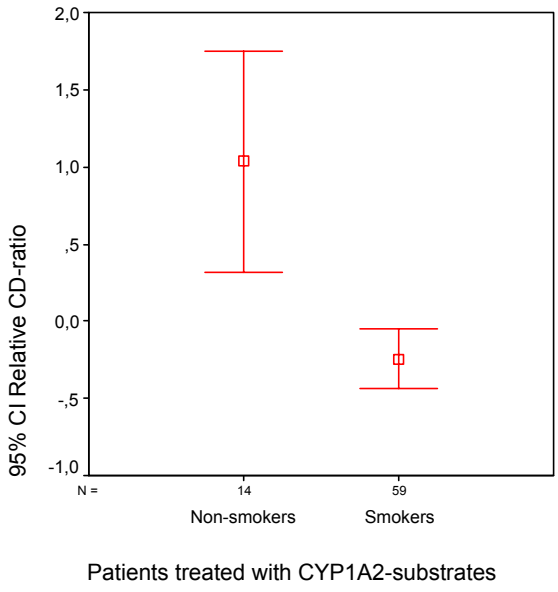

***Fig 3-7:** 95% CI for CD-ratios in all CYP1A2 users (olanzapine/clozapine), denoted as SD from average value*

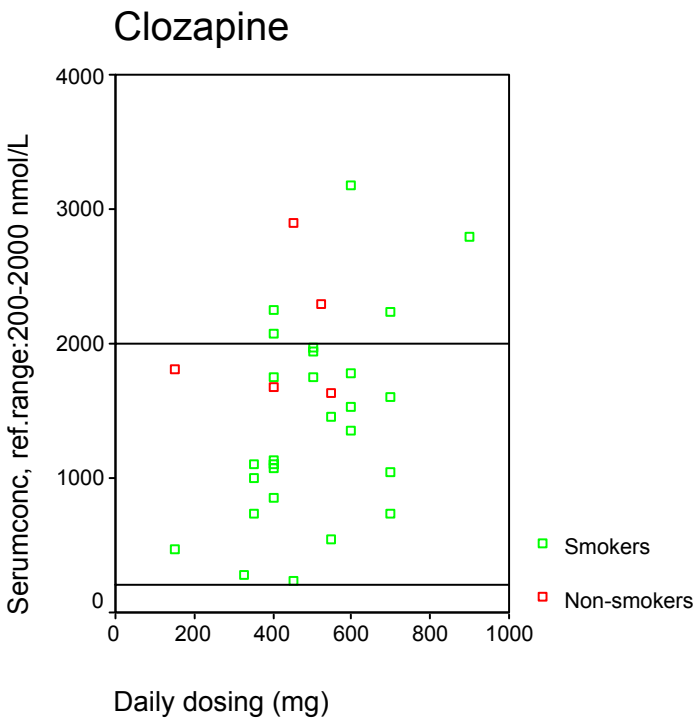

Fig 3-8: *Dosing and serum concentrations in smokers and non-smokers treated with clozapine*

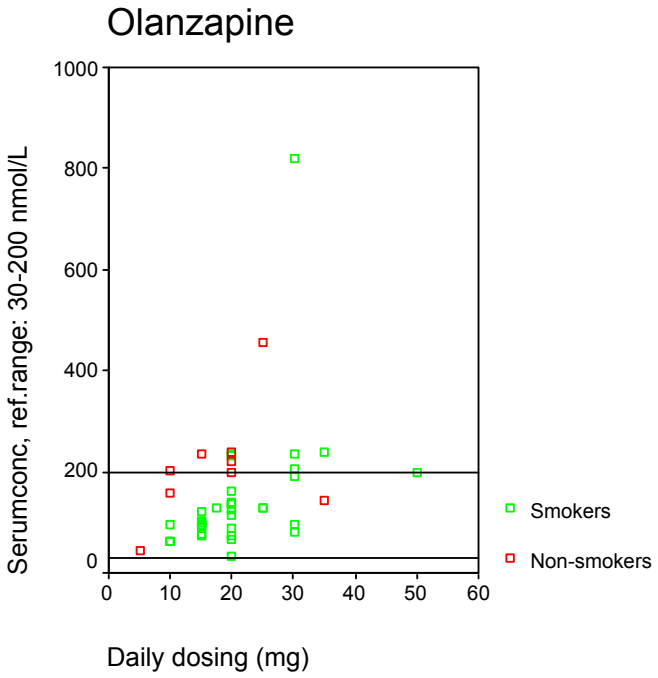

Fig 3-9: *Dosing and serum concentrations in smokers and non-smokers treated with olanzapine*

4 DISCUSSION

4.1 Patient group

This study describes a group of patients with long-term mental illness (31 years on an average) that has not responded well to treatment. In contrast most of the published studies in this field have focused on short time hospitalized patients or outpatients with a shorter psychiatric history. Age of disease onset in this study was 24 years, reflecting literature values for age of onset 16-30 years (Mueser and McGurk).

The everyday lives of these patients are characterized by established routines for taking medication, meals, activities and sleep. Non-compliance or use of alcohol and illegal drugs was relatively rare in this population according to the patient records. Both non-compliance and illegal use of drugs is considered a major problem in younger patients and in outpatients in general. No study has, to our knowledge, described the pharmacological treatment of a group of schizophrenic patients living in nursing homes.

4.1.1 Pharmacotherapy of schizophrenia in nursing homes

Eighty percent of the patients were treated with at least one atypical antipsychotic, with olanzapine and clozapine as the far most abundant (70% of the patients). The most recent marketed antipsychotics quetiapine, amisulpride and ziprasidone were only used in treatment of 7 patients. This may reflect a threshold for trying new substances in patients with already established long-term treatment.

Twenty percent of the patients received treatment with typical antipsychotics only. The low potency typical antipsychotics were dosed low according to WHO-defined DDD. This may be explained by utilization of the sedative effects of these antipsychotics primarily. It appears that WHO-defined DDD does not comply with current Norwegian clinical practice. Low potency typical antipsychotics are not being used as primary treatment of psychosis; rather in low doses to relieve agitation and restlessness associated with psychiatric disorders. Anxiolytic and sedative effects are pronounced in dosage far below the defined DDD. Clinical guidelines recommend the use of atypical antipsychotics as first line treatment of psychosis (Tanum).

4.1.2 Polypharmacy

The average patient received 1.5 different antipsychotic substances, with a total dose corresponding with 2.2 DDD. A Norwegian study on consumption of antipsychotics showed an average use of 1.22 DDD/day in psychiatric hospitals in year 2000 (Rytter and Haberg). If our patient group is more severely ill than an average group of psychiatric hospital patients they might need higher doses of antipsychotics. Regulation of CNS receptors might require increased dosage in long-term treatment. The high daily dose may perhaps also indicate that current guidelines and trends are not complied.

The Norwegian consumption study showed a total decrease in DDD of antipsychotic drugs during the 1990s. The use of typical antipsychotics decreased, while the use of atypical antipsychotics increased with 46% from 1991 to 2000. This trend might be explained by decreased use and dosage of the low potency typical antipsychotics. Increased use of atypical antipsychotics can be explained by marketing of several new substances, good tolerability and clinical experience that might lead to use of higher doses.

4.1.3 Concomitant medication

Psychotropic drugs

Each patient received an average of 2.7 different concomitant drugs. Psychotropic drugs, excluding antipsychotics (ATC group N) constituted 70% of the concomitant medication. This profile might be explained by a shift from utilization of the sedative effect of low potency typical antipsychotics to increased use of mood stabilizers and benzodiazepines (Tanum).

Antidepressive treatment was given to 40 patients. None of these patients were treated with the potent CYP2D6 inhibitors paroxetine, fluoxetine and fluvoxamine. Increased focus on drug interactions associated with some selective serotonin reuptake inhibitors (SSRI) might have influenced the choice of substance.

Somatic disorders and increased mortality in schizophrenic patients

Mortality in schizophrenic patients is 2-3 times higher than in the general population. Most of this is related to somatic conditions (60-70%), with increased risk of cardiovascular diseases as the main reason. Side effects of atypical antipsychotics include metabolic complications like weight gain, hyperlipidemia and a higher incidence of diabetes mellitus. These side effects are more pronounced in patients treated with clozapine and olanzapine (Brown, Inskip and Barraclough). Low activity level and heavy smoking are factors that suggest close monitoring of these patient's general health. Sixteen of the patients used drugs in ATC group C (cardiovascular drugs), a group that also include drugs for treating hyperlipidemia. The risk of high cholesterol- and serum lipid values are five times higher in patients treated with olanzapine (Koro et al.). An epidemiological study showed that 7% of 60-year-old people in Oslo were treated for high cholesterol levels in year 2000 (Sogaard et al.). In our patient group (55-year-old) only five patients (4.6%) were treated with statins. This might indicate room for improvement in concomitant drug treatment.

4.2 Role of CYP450 in metabolism of psychotropics

Pharmaceutical industry has increased their focus on CYP-enzymes in research and development of new drugs. Risk of drug interactions and impact on CYP metabolism has contributed to avoid new substances that inhibit or modify specific CYP-enzymes. The fact that most of the psychotropics are metabolized by CYP-enzymes emphasizes the need for knowledge about this enzyme system. Personnel involved in psychopharmacotherapy should acquire knowledge about the most important groups of inducers and inhibitors.

4.3 Use of CYP-genotyping

Only one out of 109 patients had been genotyped prior to the project, indicating that CYP genotyping is not performed on a regular basis in this group of patients. The use of CYP-genotyping is growing in the psychiatric field. Patients in need of long-term antipsychotic medication need a well-designed drug regime supported by an initial genotyping. A potential lifelong treatment with drugs suggests that an initial genotyping can be cost-effective. Pharmacogenetic analyses may contribute to reduce adverse drug reactions and lack of drug effect by predicting possible pharmacological pitfalls. The optimal dosing will be highly variable among patients depending on genotype. In addition to genetic variability, the

situation is made even more complex by environmental factors, other enzyme systems and transporters. Today genotyping requires special competence. However the technology has been simplified during the recent years. CYP genotyping may contribute to achieve a safer and more rational pharmacotherapy. A consensus committee might be required to work out guidelines to suggest an optimal use of genotyping and how to interpret information from such findings. The cost of genotyping has been considered to be high, but equals only one day of hospitalization or one injection of Risperdal Consta® (risperidone administrated every two weeks). Poor response of antipsychotic drug treatment and the development of adverse drug reactions can lead to patient non-compliance, psychosocial disturbances and poor outcome. Cost-effectiveness of TDM and genotyping has not yet been proved in large scale, prospective studies.

4.3.1 CYP-genotype in the patient group compared to the control group

No significant differences were found between the patient group, control group, and allele frequencies found in the literature. Schizophrenic patients appear to have the same CYP-genotype as the Caucasian Norwegian population. The control group differed from the patient group in respect to gender and age. These factors were not considered to influence the genotype of the population (Schwartz). Poor response or high frequency of side effects in pharmacotherapy of psychiatric patients is probably not caused by higher incidence of poor- or ultra rapid metabolizer genotype. The frequency of relevant CYP-mutations are high, 40% of the patients had a mutation in CYP2D6, the isoenzyme involved in metabolism of 60% of the prescribed antipsychotics in this population.

4.4 Impact of CYP genotype

4.4.1 Ultra rapid metabolizer

Only four patients (3.6%) were positive for the CYP2D6 *2 mutation, coding for gene duplication. Three of these patients received treatment with CYP2D6 substrates. However, the gene duplication appeared only to have clinical importance in one of the patients, demonstrated by sub-therapeutic serum concentrations. The PCR method used in this project did not determine the number of active genes, which previous studies have reported to be as high as 13 copies (Johansson et al.). The Scandinavian Caucasian population represent one of

the populations with lowest prevalence of ultra rapid metabolizers (UM) in respect to CYP2D6.

Studies have shown that as much as much as 5.5% of the European population carry the UM genotype with the following distribution pattern: 1-2% in Scandinavia; 3.6% in Germany; 7-10% in Spain, 10% in Italy and Sicily. Studies have further shown an occurrence in Saudi Arabians and Ethiopians of 20% and 29% respectively (Bertilsson et al.). With an increasing immigration from the Middle East and Africa to the Norwegian society, the share of ultra rapid genotype will probably increase over time.

4.4.2 Poor metabolizers

Five out of 109 patients (4.6%) tested positive for the CYP2D6 poor metabolizer (PM) genotype. Our data indicated higher relative exposures of CYP2D6 substrates in the PM group. When such a genotype is discovered in a patient, alternative antipsychotics not metabolized by CYP2D6 should be considered. An alternative approach is titration with low dosing of a CYP2D6 substrate in combination with therapeutic drug monitoring. One of the patients in the project with PM-genotype was treated with 0.2 DDD risperidone, resulting in serum concentrations corresponding to mid reference range.

4.4.3 Intermediate metabolizers (heterozygote extensive metabolizers)

Intermediate metabolizers showed slightly higher relative concentration to dose ratios (CD 1.3) compared to the group extensive metabolizers (normal). This indicates decreased CYP2D6 clearance. The variability in the group is high (SD 1), which suggests close follow-ups of IM genotype patients on pharmacological treatment. Thirty percent of the patients (n=33) was intermediate metabolizers for CYP2D6, twenty of these was treated with CYP2D6 substrates. IM genotype does not necessarily affect clearance in monotherapy, but might be relevant in patients treated with more than one CYP-substrate or concomitant use of CYP-inhibitors. This group of heterozygote extensive metabolizers has not been statistically explored due to multiple factors influencing the relevance of genotype.

4.5 Impact of CYP inhibitors and CYP inducers

4.5.1 Impact of CYP inhibitors

Use of CYP inhibitors did not change the average serum concentrations of antipsychotic treatment in our group. Average dosing of the CYP-inhibitors were low compared to DDD and may explain the relative lack of inhibition. Findings in our study do not support that levomepromazine and perphenazine are potent inhibitors of CYP2D6 in low doses. Yoshimura and coworkers reported that levomepromazine was not a not a relevant CYP-inhibitor in low doses (Yoshimura, Ueda and Nakamura). Adaptation and regulation of enzyme activity might contribute to the low observed relevance of CYP inhibition. No differences were found between perphenazine and levomepromazine in relation to CYP-inhibition.

4.5.2 Impact of CYP inducers

Five patients received treatment with the potent CYP-inducer carbamazepine, but only three of them were treated with antipsychotics metabolized by isoenzymes prone to induction. One of the patients showed a serum concentration far below therapeutic range, and the two other patients had twofold relative CD ratios. Drug-to-drug interactions with carbamazepine seemed not to be taken into consideration when treatment was decided. Our data suggest that treatment with carbamazepine requires close therapeutic drug monitoring of CYP-substrates.

4.6 Impact of cigarette smoking on CYP1A2

4.6.1 Impact of smoking on serum concentrations of CYP1A2 substrates

Eighty percent of the patients treated with CYP1A2 substrates (clozapine or olanzapine) smoked cigarettes. The minimum consumption was 8 cigarettes daily, indicating induction of CYP1A2 by polyaromatic hydrocarbons. The non-smokers had two-fold higher CD ratios of CYP1A2 substrates compared to smokers. Our findings are supported by earlier studies showing a 2.5 fold higher CD ratios in non-smokers compared to smokers on long term clozapine therapy (van der Steijns and van Weelden). Smokers showed 30% decrease in both clozapine (2060nmol/L vs. 1406nmol/L) and olanzapine (210nmol/L vs. 147 nmol/L) serum concentration. Studies have shown a 20-40% decrease in serum concentration from the same dose of clozapine in smokers compared to non-smokers (Meyer); (Seppala et al.).

The smokers received higher doses of clozapine and olanzapine than non-smokers. However it appears that smoking behavior did not closely correlate to dosing of antipsychotic. This raises the question if there is a tendency to underdose smokers and overdose non-smokers, since this issue is apparently not a focus in clinical practice. Health care personnel should be aware of changes in smoking behavior, which may lead to substantial changes in serum concentration of drugs metabolized by isoenzyme CYP1A2 (Zullino et al.). It has been suggested that doses of CYP1A2 substrates should be decreased immediately on cessation of heavy smoking. A dose reduction of 10% every day until the fourth day was recommended together with TDM (Faber and Fuhr). This implies regularly monitoring of smoking behavior in patients treated with CYP1A2 substrates.

Another intriguing aspect is the lack of close correlation between number of cigarettes and variation in serum concentrations. When comparing different subgroups of smokers, no significant differences were found between light and heavy smokers in relation to apparent CYP1A2 activity. Regression of the concentration to dose ratio and number of cigarettes revealed a Pearson Correlation of –0.11 ($p=0.44$), which only indicates a minor relationship between higher CYP1A2 clearance in heavy compared to light smokers ($n=63$). For the clinician it does not seem crucial to obtain the precise consumption of cigarettes, just establish if the patient smokes cigarettes or not. These findings should be further explored due to the potential clinical impact and because of a low number of subjects in the non-smoker group.

4.6.2 Individual variability

In this project bivariate correlation barely showed significant connection between daily dosing and serum concentrations in clozapine ($p=0.042$, Pearson 0.36) and olanzapine ($p=0.024$, Pearson 0.35). This confirms that multiple factors are important in predicting the response of antipsychotic treatment. The degree of environmental and genetic variability depends on the CYP isoenzyme involved.

4.7 Use of CYP genotyping and TDM in psychiatry

Studies have shown that the period of untreated schizophrenia is important in the course of the disorder. Psychosis and schizophrenia are often untreated for a period of two-three years (including the prodromal phase) (Melle et al.). Initial assessments, testing and individualization of pharmacological treatment might help decreasing the lag time to therapeutic effect. TDM should be performed several times in the establishment and adjustment of pharmacological regime. Evaluation of genotyping and TDM-results require knowledge about metabolism, enzyme systems and pharmacokinetics. Patient information, genotype and serum concentrations have to be interpreted together to obtain valuable information. Genotyping alone will not provide this information, but used in combination with TDM it might be a step towards individualized medicine. There seems to be a lack of knowledge of drug monitoring and genotyping amongst a number of prescribing physicians. Increased use of these tools might reduce the current use of "trial and error" practice in optimizing psychiatric pharmacological treatment.

4.8 Limitations of the study

The patients were not assessed with diagnostic tools (e.g. PANSS) by the investigators. Diagnoses were obtained from patient records deriving from previous or current psychiatric hospitals, institutions or nursing homes. The diagnostic work had been performed by a wide range of physicians over a long period of time.

For a number of parameters we found a rather high degree of variability, which made the use of advanced statistical tools inappropriate. Small groups with unequal distribution complicated the work of detecting possible relationships between clinical factors and pharmacotherapy. This led to the descriptive and explorative nature of this study.

Time from last dose of drug is a crucial factor in measuring serum concentrations. Reference ranges are based on trough-values, because the period prior to a new dose shows least amount of fluctuations in serum concentrations. Serum concentrations were only measured once, and the possibility of non-compliance or variation in the time from last dose cannot be ruled out.

4.9 Concluding remarks

Schizophrenic patients on long-term treatment in nursing homes receive today a rather comprehensive drug treatment. Polypharmacy both in respect to antipsychotic and concomitant medication was common. This group of patients appears to be dosed higher than other patients in psychiatric hospitals.

Multiple factors influence the CYP-activity of the patients; this includes drug-interactions, genetic polymorphism and cigarette smoking. All these factors have to be considered in psycho-pharmacotherapy. Isolated, certain drug-interactions and intermediate metabolizer genotype appeared to be clinically relevant only in some of the patients in our study. Several factors need to be taken into consideration in explanation of serum concentrations from a given dose of drug. Genetic and environmental factors have different impact on the different CYP isoenzymes. The most important isoenzymes in this group of patients are CYP1A2 and CYP2D6. Both of them are relevant in metabolizing antipsychotics in approximately two-thirds of the patient. CYP2D6 exhibit clinically relevant genetic variation. CYP1A2 does not exhibit genetic variations, but is prone to induction from polyaromatic hydrocarbons from cigarette smoking. The fact that 75% of these patients smoke cigarettes and 40% carry CYP2D6-mutations should emphasize focus on the CYP-system in psychiatry.

Increased knowledge and availability of CYP-genotyping and therapeutic drug monitoring represents an opportunity to reassess the pharmacotherapy of chronically ill schizophrenic patients in nursing homes.

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