Pharmacogenetics and Antipsychotic Treatment in Schizophrenia with Special Focus on Adverse Drug Reactions

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Abstract

Genetically determined differences in drug metabolism and disposition and drug targets play a pivotal role in the interindividual variability in the clinical outcome of antipsychotic treatment. The aim of this thesis was to study the impact of polymorphisms in genes involved in the pharmacokinetics and pharmacodynamics of antipsychotics, with special focus on their extrapyramidal and metabolic adverse effects.

Polymorphisms in serotonin 2A and 2C receptor coding genes (HTR2A and HTR2C) were found to be associated with the risk to develop extrapyramidal side effects (EPS) in patients on short term perphenazine treatment. A further study in a larger group of patients on long term treatment with various classical antipsychotics confirmed the association between occurrence of EPS and HTR2C polymorphisms. In another study, dose corrected steady state serum clozapine and N-desmethylclozapine concentrations (C/D) and insulin elevation during clozapine therapy were found to correlate with CYP1A2 but not with CYP2D6 polymorphisms. Furthermore, HTR2C and HTR2A polymorphisms were found to have significant influences on BMI and C-peptide levels in patients treated with olanzapine and clozapine. Evaluation of the impact of polymorphisms in genes encoding CYP3A4, CYP3A5 and P-glycoprotein (ABCB1) in addition to CYP2D6 on the steady state plasma levels of risperidone, 9-hydroxyrisperidone and their active moiety revealed a significant influence of ABCB1 genotype on 9-hydroxyrisperidone and active moiety C/Ds, while CYP2D6 genotype associated with risperidone C/Ds but not with 9-hydroxyrisperidone or active moiety C/D.

We have shown that polymorphisms in genes involved in the pharmacokinetics and the pharmacodynamics of antipsychotic drugs play a role in the occurrence of adverse effects, both EPS and metabolic disturbances, induced by antipsychotic treatment. Genotyping for HTR2A, HTR2C, CYP1A2, CYP2D6 and ABCB1 polymorphisms may therefore potentially provide useful information to identify patients at higher risk to develop EPS or metabolic adverse during schizophrenia treatment with antipsychotic drugs.

Keywords: Pharmacogenetics, schizophrenia, antipsychotic treatment, adverse effects, clinical outcome

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“Little by little, one travels far.”

*J.R.R. Tolkien* (1892-1973)
List of papers


IV **Gunes A**, Melkersson KI, Scordo MG, Dahl M-L. Association between HTR2C and HTR2A polymorphisms and metabolic abnormalities in patients treated with olanzapine and clozapine. *(Manuscript)*

V **Gunes A**, Spina E, Dahl M-L, Scordo MG. ABCB1 polymorphisms influence steady state plasma levels of 9-hydroxyrisperidone and risperidone active moiety. *(Submitted to Therapeutic Drug Monitoring)*

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Abbreviations

**ABCB1** Adenosine triphosphate-binding cassette B1 coding gene
AIMS Abnormal involuntary movement scale
BAS Barnes akathisia rating scale
BMI Body mass index
C/D Concentration to dose
CI Confidence interval
CYP Cytochrome P450
dNA Deoxyribonucleic acid
DSM-IV Diagnostic and statistical manual of mental disorders
D2 Dopamine receptor type 2
D3 Dopamine receptor type 3
**DRD2** Dopamine receptor D2 coding gene
**DRD3** Dopamine receptor D3 coding gene
EM Extensive metabolizer
EPS Extrapyramidal side effects
FMO Flavin-containing monooxygenase
HPLC High performance liquid chromatography
HOMA-IR Homeostasis model assessment index for insulin resistance
LD Linkage disequilibrium
P-gp P-glycoprotein
PCR Polymerase chain reaction
PM Poor metabolizer
PANSS Positive and negative symptom scale
RFLP Restriction fragment length polymorphism
5-HT Serotonin
5-HT_{2A} Serotonin receptor type 2A
5-HT_{2C} Serotonin receptor type 2C
**HTR2A** Serotonin receptor type 2A coding gene
**HTR2C** Serotonin receptor type 2C coding gene
SAS Simpson-Angus scale
SNP Single nucleotide polymorphisms
UGT Uridine diphosphate glucuronosyltranferase
UM Ultrarapid metabolizer
Introduction

Schizophrenia treatment

Schizophrenia is a debilitating mental disorder with onset usually in early adolescence or early adulthood and with 1% life time prevalence [1]. The symptoms of schizophrenia are classified as positive (delusions, hallucinations, disorganized speech or thinking, catatonic behaviors), negative (flattening of the affect, poverty of thought or speech, apathy, social withdrawal) and cognitive (distractibility, impaired memory and executive function).

Dopaminergic and serotoninergic pathways are involved in the etiology of schizophrenia and in the clinical outcome of antipsychotic drug therapy, in both therapeutic and adverse effects. Dopaminergic hyperactivity in the mesolimbic pathway is suggested as the main mechanism leading to positive symptoms [2], while hypoactivity in mesocortical dopaminergic pathway seems to be associated with negative symptoms and impaired cognition [3].

Antipsychotic drugs are the basis of schizophrenia treatment. Patients generally need a life-long therapy. However, the clinical outcome of antipsychotic treatment shows a large interindivudal variability. Only about half of the patients benefit from the treatment, while the rest experience either adverse effects or therapeutic failure [4-6].

Current antipsychotic drugs

The first generation “classical” antipsychotic drugs (e.g. chlorpromazine, haloperidol, perphenazine, thiothixene) are characterized by high dopamine D$_2$ receptor antagonistic affinity, whereas the second generation so called “atypical” antipsychotic drugs (e.g. clozapine, olanzapine, risperidone, ziprasidone, aripiprazole) have lower antagonistic affinity for dopamine D$_2$ receptor, and higher affinity for serotonin (5-HT) receptors together with histaminergic, muscarinic, and adrenergic receptor affinity [7, 8].

The major disadvantage of the treatment with classical antipsychotics is the occurrence of extrapyramidal side effects (EPS) and prolactin elevation also at therapeutic doses. The lower propensity of atypical antipsychotics to cause EPS and prolactin elevation has provided clinical advantages. However, weight gain and related metabolic adverse effects are reported more
frequently during treatment with atypical antipsychotic drugs and have ra-
ised clinical concern [5, 9].

A recent study, evaluating the clinical outcome of a number of antipsy-
chotic drugs after 18 months treatment in 1432 chronic schizophrenic pa-
tients, showed that the classical antipsychotic perphenazine had similar effica-
cy as some of the atypical antipsychotics including risperidone, ziprasidone
and quetiapine [4]. However, discontinuation rates were high in the perphe-
nazine treated group due to EPS and in the olanzapine treated group due to
weight gain. This suggests that both classical and atypical antipsychotic
drugs play important roles in the treatment of schizophrenia, but differ in
their adverse effect profiles and patient tolerability.

Perphenazine
Perphenazine is a phenothiazine derivative with similar antipsychotic poten-
cy as haloperidol. It has high antagonistic affinity for dopamine D₂ receptors,
and weak 5-HT₂A, dopamine D₄ and muscarinic M₁ receptor antagonistic
affinities [10]. The N-dealkylation of perphenazine to its 7-hydroxy metabo-
lite has been shown to be catalyzed mainly by CYP2D6 in vivo [11] and in
vitro, with minor contributions of CYP1A2, CYP3A4 and CYP2C19 [12].

Clozapine
The prototype of the atypical antipsychotic drugs clozapine is a dibenzidia-
zepine derivative with a low antagonistic affinity for D₂ receptors and a high
affinity for serotonergic 5-HT₂A, 5-HT₂C, 5-HT₁A, histaminergic H₁, dopa-
mnergic D₁-5, adrenergic α₁ and α₂, and muscarinic M₁ receptors [13]. Clo-
zapine is highly effective against both positive and negative symptoms in
patients not responding or intolerant to other antipsychotics. It has a low
propensity to induce EPS and prolactin elevations [14]. However, granulocy-
topenia and agranulocytosis are serious adverse effects of clozapine, obser-
vied in 1-2% of the patients and the use of clozapine is therefore limited [15].
The metabolism of clozapine to its active N-desmethyl metabolite is cataly-
zed by CYP1A2, CYP2D6, CYP2C19 and CYP3A4 in vitro, while CYP3A4
and FMO3 mediate its N-oxidation [16, 17]. The glucuronidation of clozapi-
ne is catalyzed by the UDP-glucuronosyltransferases 1A3/4 (Figure 1) [18].

Similar to clozapine, N-desmethyleclozapine has been reported to have a
high affinity for the 5-HT₁A and 5-HT₂ receptors in vitro [19]. Steady state
plasma concentrations of clozapine correlate with both therapeutic and ad-
verse effects [20] and show up to 45-fold interindividual variation [21].
Olanzapine
Olanzapine is used in the treatment of schizophrenia for its ability to improve both negative and positive symptoms. It has a broad receptor affinity profile including antagonistic effects for serotonin 5-HT\textsubscript{2A/2C}, 5-HT\textsubscript{3}, 5-HT\textsubscript{6}, dopamine D\textsubscript{1,5}, muscarinic M\textsubscript{1}, adrenergic α1 and histaminergic H\textsubscript{1} receptors [20, 22]. Olanzapine is mainly metabolized via N-glucuronidation mediated by UGT1A4 and via N-demethylation mediated by CYP1A2, whereas 2-hydroxylation via CYP2D6 and N-oxidation via FMO3 are minor metabolic pathways [23-25]. Olanzapine has a high propensity to induce metabolic adverse effects [4, 26].

Risperidone
Risperidone is a very potent 5-HT\textsubscript{2A} antagonist, showing 20 times higher antagonistic affinity for 5-HT\textsubscript{2A} receptors than for dopamine D\textsubscript{2} receptors [14, 27]. The antipsychotic effect of risperidone is assumed to be related to the sum of risperidone and its active metabolite, 9-hydroxyrisperidone, defined as the active moiety. 9-hydroxyrisperidone has a similar receptor binding profile as risperidone and constitutes the major part of the active moiety in plasma [28, 29]. The risk for EPS increases at higher doses as well as at higher plasma levels of risperidone active moiety [30, 31]. The metabolism of risperidone to 9-hydroxyrisperidone in the liver is catalyzed mainly by CYP2D6 and CYP3A4 [32-34].
Antipsychotic induced adverse effects

Adverse drug effects such as EPS and metabolic disturbances constitute an important drawback for patient safety and compliance to the therapy. Treatment failure, relapse of psychosis, increased metabolic and cardiovascular morbidity and mortality are potential consequences of these adverse effects [35, 36].

EPS may occur in up to 90% of patients within days or years of antipsychotic treatment [37, 38]. The most common EPS are dystonia (involuntary muscular spasms, abnormal postures including oculogyric crisis, tongue protrusion, trismus, torticollis, laryngeal-pharyngeal constriction, bizarre positions of limbs and trunk), parkinsonism (tremor, rigidity, bradykinesia), akathisia (a subjective feeling of restlessness, agitation, repetitive purposeless actions), and tardive dyskinesia (persistent involuntary movements, especially around the mouth). In clinical practice, parkinsonism and related EPS are generally assessed by the Simpson-Angus Scale (SAS) [39], akathisia by the Barnes Akathisia Rating Scale (BAS) [40] and tardive dyskinesia by the Abnormal Involuntary Movement Scale (AIMS) [41].

Metabolic adverse effects show a large interindividual variation in severity, from weight gain, glucose intolerance, lipid abnormalities and hypertension to type 2 diabetes mellitus, diabetic coma and death [42, 43]. Both classical and atypical antipsychotics may cause metabolic disturbances; however, olanzapine and clozapine have been most frequently associated with these adverse effects [4, 26, 42, 44].

Nigrostriatal dopamine D2 receptor antagonism and compensatory dopamine receptor supersensitization have been hypothesized as underlying mechanisms for motor disturbances induced by antipsychotic drugs [45, 46]. The serotonergic system has an inhibitory control on the nigrostriatal dopaminergic transmission [47]. Thus, additional to dopaminergic receptors, serotonergic receptors may also play a role in the development of EPS.

The serotonergic system is also involved in the regulation of feeding behavior and satiety control in the central nervous system together with multiple neurohormonal regulatory pathways, including leptin, neuropeptide Y, pro-opiomelanocortin, ghrelin and orexin [48, 49]. Although the mechanisms underlying the metabolic adverse effects of antipsychotic drugs are unclear, 5-HT2A and especially 5-HT2C receptor antagonism is suggested to lead to increased appetite and weight gain [50-52]. Partial agonism of 5-HT1A and antagonism of histamine H1, adrenergic β2, β3 and α1 receptors are also implicated as possible pharmacological mechanisms for weight gain induced by antipsychotic drugs [53-55]. Furthermore, a direct effect of the drugs on basal insulin release from isolated pancreatic islets has been shown in vitro for olanzapine and clozapine [56, 57]. Moreover, the levels of insulin and triglycerides during clozapine treatment were found to be correlated with serum concentrations of the drug [58].
The role of pharmacogenetics in the treatment of schizophrenia

Identification of patients who are likely to respond to the therapy or prone to develop adverse effects may help clinicians to avoid treatment failure and to prevent patient morbidity. Pharmacogenetic studies aimed to evaluate genetic factors contributing to the interindividual variation in clinical outcome of antipsychotic treatment have been focusing on two main aspects so far: genetic variations (insertions, deletions, single nucleotide polymorphisms and copy number variations in the DNA sequence) of relevance for either the pharmacokinetics or the pharmacodynamics of the drugs (Figure 2).

Significant associations between polymorphisms in the genes coding for drug metabolizing enzymes as well as serotonin and dopamine receptors and clinical outcome of antipsychotic therapy in a number of studies, but the results have not always been confirmed [59-63].

Among many other candidate genes, catechol-O-methyl transferase (COMT), monoaminooxidase A (MAOA), monoaminooxidase B (MAOB), histamine H1 receptor, β3 adrenergic receptor, α1 and α2 adrenergic receptor, leptin receptor, pro-opiomelanocortin (POMC), tumor necrosis factor alpha (TNF-α) and synaptosome-associated protein of 2500 daltons (SNAP 25) coding gene polymorphisms have also been studied for their possible impact on the therapeutic and adverse effects of antipsychotics [6, 53, 64, 65].

The studies included in this thesis focus on the polymorphisms in the pharmacokinetic related genes CYP1A2, CYP2D6, CYP3A4/5, P-glycoprotein (ABCB1) and in the antipsychotic drug targets, serotonin and dopamine receptor coding genes (HTR2A, HTR2C, DRD2 and DRD3).
Genetic variation in drug metabolism and disposition

Metabolic pathways of antipsychotic drugs include oxidation, reduction, hydrolysis (Phase I) reactions as well as conjugation reactions via glucuronidation (Phase II). The majority of phase I reactions are catalyzed by cytochrome P450 (CYP) enzymes. The main CYP enzymes involved in the metabolism of antipsychotic drugs are CYP1A2, CYP2D6 and CYP3A4 [66, 67].

A large interindividual variability exists in drug metabolism, partially due to the genetic variability in genes coding CYP enzymes. Subjects who carry 1 or 2 copies of a functional allele have a normal metabolic activity and are classified as extensive metabolizers (EM), while those carrying two detrimental alleles lack the enzyme activity and are defined as poor metabolizers (PM). Moreover, in the case of CYP2D6, subjects carrying more than two copies of a functional allele exhibit a high metabolic rate for the substrates of the enzyme and are classified as ultrarapid metabolizers (UM) [68]. A decreased metabolic capacity may lead to high plasma levels and increased risk of toxicity or, if the main compound is a pro-drug that needs to be activated, to therapeutic failure. On the other hand, being an UM may lead to low plasma levels of the drug causing therapeutic failure.

CYP1A2

Clozapine and olanzapine are to a major extent metabolized by the CYP1A2 enzyme [66]. The metabolic activity of this enzyme shows large interindividual variation, due to genetic as well as environmental factors [20]. Smoking is a potent inducer of CYP1A2 activity and several drugs, including fluvoxamine and fluoroquinolons, inhibit its activity [69, 70].

The CYP1A2 gene, located on chromosome 15 [71], is highly polymorphic (http://www.cypalleles.ki.se/cyp1a2.htm). A few common CYP1A2 polymorphisms have been studied intensively. However, the impact of these polymorphisms on enzyme activity is still not totally clear.

- **-3860G/A (rs2069515) (CYP1A2*1C) polymorphism** in the flanking region of the gene leads to a decreased inducibility of the enzyme in smokers most probably due to a decreased expression of the enzyme [72].
- **-2467delT (rs35694136) (CYP1A2*1D) 5’-flanking region polymorphism.** No clear influence of this polymorphism has been shown on the enzyme activity [72-74].
- **-163C/A (rs762551) (CYP1A2*1F) polymorphism** in intron 1, was reported to cause high enzyme inducibility in smokers [72-75].
- **CYP1A2*1K (-163A, -739G (rs2069526), -729T (rs12720461)) haplotype was found to be related to reduced CYP1A2 activity compared to CYP1A2*1A (wild type) and CYP1A2*1F (-163A) or CYP1A2*1J (-163A, -739G) haplotypes in non-smoker volunteers [76].**
An association between the *CYP1A2* genotype and plasma levels as well as therapeutic response to clozapine treatment has been shown [77, 78]. Recently, *CYP1A2* -163C/A and -3860G/A polymorphisms have been evaluated for a possible association with olanzapine and N-desmethylolanzapine plasma levels and no significant association was found [79].

**CYP2D6**

Several antipsychotics including perphenazine, chlorpromazine, thioridazine, haloperidol, zuclopenthixol and risperidone are metabolized by CYP2D6 [11, 66, 80]. Unlike CYP1A2, CYP2D6 activity is not inducible, but a number of drugs, including paroxetine, fluoxetine and quinidine inhibit its activity [66, 81]. There is a large interindividual variation in CYP2D6 activity, determined mostly by genetic polymorphisms [67].

The *CYP2D6* gene is located on chromosome 22. Over 60 allelic variants have been described (http://www.cypalleles.ki.se/cyp2d6.htm).

- *CYP2D6*3, *CYP2D6*4, *CYP2D6*5 and *CYP2D6*6 variants are associated with complete lack of enzyme activity, leading to PM phenotype.
- *CYP2D6*1XN and *2XN*, the duplication or multiplication of a functional *CYP2D6* gene causing extremely high CYP2D6 activity and leading to UM phenotype.

The PM phenotype among Caucasians has a frequency of 3-10%, and the UM of 1-10% [67, 82]. The CYP2D6 genotype has been reported to predict the oral clearance of perphenazine, zuclopenthixol and haloperidol [83, 84]. Moreover, an increased tendency to develop various EPS in CYP2D6 PMs has been reported [85, 86]. Previously, the *CYP2D6* genotype has been shown to have a significant impact on steady state plasma levels of risperidone, but not on those of 9-hydroxyrisperidone or the active moiety [87].

**CYP3A4/5**

The CYP3A4 enzyme is involved in the metabolism of e.g. haloperidol, bromperidol, quetiapine and risperidone [6, 66]. The expression of CYP3A enzymes can be induced by environmental factors including drugs such as rifampicin and carbamazepine, and inhibited by macrolide antibiotics, anti-HIV agents and grapefruit juice [88]. Genetic factors are also expected to influence the activity of the enzymes [89]. A number of allelic variants of *CYP3A4* (http://www.cypalleles.ki.se/cyp3a4.htm) and *CYP3A5* (http://www.cypalleles.ki.se/cyp3a5.htm) have been described. However, whether they influence the clinical outcome of antipsychotic treatment has not been reported yet.
• \textbf{CYP3A4*1B (rs2740574)} is in the promoter region of the gene potentially affecting the transcription efficiency and thus the overall enzymatic activity of CYP3A4 [90].

• \textbf{CYP3A4*3 (rs4986910)} leads to a Met445Thr substitution near the active site of the enzyme causing altered substrate specificity [91].

• \textbf{CYP3A4*4} variant is characterized by an Ile118Val substitution, and a decrease in enzyme activity [92].

CYP3A4 and CYP3A5 enzymes share substrate specificity [93]. Only subjects carrying at least one functional \textit{CYP3A5*I} allele express the CYP3A5 protein [94].

• \textbf{CYP3A5*3 (rs776746)} polymorphism, leading to alternative splicing of CYP3A5 transcript and absence of the protein, is the main cause of the absence of CYP3A5 expression in more than 70% of Caucasians [94].

• \textbf{CYP3A5*2 (rs28365083)} and \textbf{CYP3A5*6 (rs10264272)} code for an enzyme without activity [95].

\textbf{P-glycoprotein}

P-glycoprotein (P-gp) is a member of the adenosine triphosphate-binding cassette (ABC) superfamily of transport proteins, acting as an efflux pump involved in drug absorption and elimination [96]. P-gp is expressed in several organs, including the apical membranes of epithelial cells in the gastrointestinal tract, the biliary canalicular membranes of hepatocytes, the luminal membranes of proximal tubular epithelial cells in the kidney and endothelial cells in the cerebral capillaries forming the blood brain barrier [96, 97]. It has been reported that risperidone and quetiapine are high affinity substrates of P-gp \textit{in vitro} [98]. However, the clinical significance of the genetic variability in P-gp function is still under debate.

The P-gp coding gene, \textit{ABCB1}, is highly polymorphic [99, 100]. The polymorphisms in exon 12, 21 and 26 have been studied most extensively.

• \textbf{1236C/T (rs1128503)} a silent polymorphism in exon 12, is in close linkage disequilibrium with the 2677G/T/A and 3435C/T polymorphisms [101, 102].

• \textbf{2677G/T/A (rs2032582)} in exon 21 has been reported to alter expression and activity of P-gp [103-105].

• \textbf{3435C/T (rs104542)} a silent polymorphism in exon 26 has been reported to be associated with lower P-gp function and duodenal P-gp expression in subjects with 3435T/T genotype compared to those with the 3435C/C genotype [106].
The reports concerning the influence of *ABCB1* polymorphisms on risperidone and 9-hydroxyrisperidone concentrations have been controversial [107-109]. However, a greater clinical improvement was reported in risperidone treated Chinese patients who carried the 1236T/T genotype [110].

**Genetic variation in dopaminergic and serotonergic receptors**

**Dopamine D\(_2\) receptor**

Dopamine D\(_2\) receptors belong to the G-protein coupled receptor superfamily and are mainly expressed in the striatum, cortex and limbic system [111]. Antipsychotic drugs have antagonistic affinity for these receptors. PET (positron emission tomography) studies showed that antipsychotic doses leading to 65% occupation of dopamine D\(_2\) receptors are enough to achieve reduction in symptoms, while over 72% occupation induces prolactin elevation and 77% or higher occupation induces EPS [30]. The dopamine D\(_2\) receptor gene, *DRD2*, is located on chromosome 11 and contains a number of SNPs.

- **-141C Ins/Del (rs1799732)** promoter polymorphism leads to a reduced promoter activity *in vitro* [112]. The -141C Del allele was found to be associated with high striatal dopamine receptor density in healthy volunteers [113].
- **Taq1A (rs1800497)** polymorphism is characterized by a C to T conversion at position 32806 of the gene. The Taq1A1 allele has been reported to lead to reduced striatal D\(_2\) receptor binding [113, 114].
- **Ser311Cys (rs1801028)** polymorphism is located in exon 7 of the gene and postulated to modulate receptor-G protein interaction and alter receptor function [115].

**Dopamine D\(_3\) receptor**

The localization of the dopamine D\(_3\) receptors is concentrated in mesolimbic areas in the brain [116]. Also coupled to inhibitory G-protein, these receptors are targeted by several antipsychotics [117]. The dopamine D\(_3\) receptor coding gene, *DRD3*, is located on chromosome 3.

- **Ser9Gly (rs6280)** polymorphism leading to a serine to glycine substitution in the N-terminal of the receptor protein, leads to a higher dopamine binding activity [118].
Previously, the DRD2 -141C Del allele was found to be associated with poor response to antipsychotic treatment [119, 120], the DRD2 Taq1A1 allele with increased EPS risk during treatment with classical antipsychotics and selective serotonin reuptake inhibitors [121, 122], and the DRD3 9Gly variant was associated with the risk of developing schizophrenia [123] and tardive dyskinesia [60].

5-HT$_{2A}$ receptor
The 5-HT$_{2A}$ receptors, coupled to stimulatory G protein, are widely expressed in cortical brain areas, additional to hypothalamus, limbic system and striatum [124]. The 5-HT$_{2A}$ receptor coding gene, HTR2A, is located on chromosome 13.

![Figure 3. HTR2A gene](image)

- -1438A/G (rs6311) promoter polymorphism leads to decreased promoter activity and lower expression of the receptor protein [125].
- 102T/C (rs6313) is a silent polymorphism within the coding region, in complete linkage disequilibrium with the -1438A/G polymorphism [126].
- His452Tyr (1354C/T) (rs6314) polymorphism causes an amino acid substitution within the cytoplasmic C-terminal tail of the receptor. The 452Tyr variant has been shown to be associated with reduced serotonin-induced calcium mobilization in platelets [127].

5-HT$_{2C}$ receptor
The 5-HT$_{2C}$ receptors are coupled to stimulatory G-protein and involved in the regulation of feeding behavior, anxiety and motor functions. These receptors are expressed in choroid plexus, prefrontal cortex, basal ganglia and limbic regions in the brain [128, 129].

The 5-HT$_{2C}$ receptor coding gene, HTR2C, is located on the X chromosome [130].
Figure 4. HTR2C gene

- **-997G/A (rs381329)** polymorphism in the promoter region is in complete linkage disequilibrium with -759C/T [131].
- **-759C/T (rs381328)** polymorphism is characterized by a C to T substitution in the promoter region of the gene, leading to decreased promoter activity [132].
- **-697G/C (rs518147)** promoter polymorphism. The -697C allele causes decreased promoter activity [132, 133].
- **Cys23Ser (68G/C) (rs6318)** polymorphism leads to a higher constitutive activity of the 23Ser compared to the 23Cys variant in vitro [134]. This finding could not be reproduced in mammalian cells expressing the mRNA edited 5-HT<sub>2C</sub> isoforms [135].

Previously, the **HTR2A** 102C allele has been found to be associated with increased risk for schizophrenia and EPS, and poor clinical response to clozapine therapy [61, 136-138]. The **HTR2C** -697C allele has been reported to be associated with persistent tardive dyskinesia [139], the **HTR2C** -759T allele with lesser weight gain induced by antipsychotic treatment [62, 140-142], and the **HTR2C** 23Ser allele with better response to clozapine treatment [143] and increased risk for tardive dyskinesia [63].
Aim

The aim of this thesis was to study the importance of polymorphisms in genes involved the pharmacokinetics and pharmacodynamics of antipsychotics, with special focus on their extrapyramidal and metabolic adverse effects.

Specific aims

- To study the impact of polymorphisms in \textit{DRD2}, \textit{DRD3}, \textit{HTR2A} and \textit{HTR2C} genes on short term clinical effects of perphenazine monotherapy.
- To evaluate the association between \textit{HTR2C} polymorphisms and the occurrence of EPS in a larger group of male schizophrenic patients treated with various classical antipsychotics.
- To study the impact of \textit{CYP1A2} and \textit{CYP2D6} polymorphisms on steady state drug serum concentrations and levels of insulin and lipids in patients treated with clozapine.
- To study the possible influence of \textit{HTR2C} and \textit{HTR2A} polymorphisms on glucose-insulin homeostasis and lipid levels in patients on long term treatment with olanzapine or clozapine.
- To evaluate the influence of \textit{CYP3A4/5} polymorphisms and \textit{ABCB1} haplotypes on the steady state plasma levels of risperidone, 9-hydroxyrisperidone and the active moiety in schizophrenic patients.
Methods

Subjects and study design

Study I
Forty-seven Estonian patients diagnosed with schizophrenia according to the Diagnostic and Statistical Manual of Mental Disorders Fourth Revision (DSM-IV) criteria were enrolled [144]. The patients (22 women, 25 men, aged 17-68 years) were treated with oral perphenazine as monotherapy (4-48 mg/day). Only benzodiazepines (22 patients) and trihexylphenidyl (4 patients) were allowed as concomitant therapy. The Positive and Negative Symptom Scale (PANSS) was used to evaluate psychotic symptoms and the SAS and the BAS scales to evaluate EPS. The evaluations were performed before starting perphenazine treatment and after 4-6 weeks of therapy, following a minimum of one week stable perphenazine dose. Patients who showed more than 20% reduction in PANSS score were defined as responders. A total score higher than three on SAS was classified as parkinsonism and a BAS score of four or higher was classified as akathisia. Blood sampling for genotyping was performed at the beginning of the study, while that for plasma perphenazine concentration analysis took place after 4-6 weeks of therapy, on the same day as the second clinical evaluation, 12±1 hours after the last dose intake.

Study II
Ninety-nine Italian, male chronic schizophrenic inpatients at Mandalari Psychiatric Hospital in Messina, Italy (aged 25-75 years) diagnosed according to DSM-IV criteria were evaluated in this study. Patients had been treated with classical antipsychotic drugs (haloperidol, perphenazine, levomepromazine, fluphenazine, chlorpromazine, thioridazine or zuclopenthixol) for at least 5 and up to 25 years (mean ± SD; 17 ± 5 years). The presence of EPS (dystonia, parkinsonism, tardive dyskinesia) was investigated with the SAS (a SAS score of 3 or more was defined as parkinsonism) and with the AIMS (an AIMS score of 4 or more indicating tardive dyskinesia) and also by reviewing the medical records of the patients.
In order to compare the distributions of \textit{HTR2C} polymorphisms between a healthy Italian population and schizophrenic patients, 112 male healthy volunteers (aged 25-50 years) resident in the same geographical area were also evaluated in the study.

\textbf{Study III and Study IV}

Study III was performed in 17 consecutive outpatients of Swedish origin (12 men, 5 women, aged 29-63 years, 6 smokers) diagnosed with either schizophrenia (n=16) or schizoaffective disorder (n=1) according to DSM-IV criteria and on therapy with clozapine.

Study IV was performed in 46 Swedish outpatients (20 women, 26 men, aged 23-63 years, 15 smokers, 31 non-smokers) with schizophrenia or schizoaffective disorder according to DSM-IV criteria and treated with olanzapine (2.5-20 mg/day, n=28) or clozapine (100-700 mg/day, n=18, including the 17 patients who had participated in study III) for at least 6 months.

Patients who had a substance-related disorder, known diabetes mellitus, other physical illness or drugs that could influence glucose or lipid metabolism were excluded. The only concomitant psychotropic drugs allowed were benzodiazepines (11 patients), levomepromazine (4 patients), and lithium (3 patients).

Fasting blood samples were collected in the morning, 12-14 hours after drug intake, for genotyping and for analysis of serum drug concentrations and metabolic parameters.

\textbf{Study V}

Forty-six patients (35 males, 11 females, aged 26 to 64 years) from Southern Italy, diagnosed with schizophrenia according to DSM-IV criteria, in therapy with risperidone (1-10 mg/day) for 4-6 weeks were included in the study. Most of the patients (n=37) had participated in a previous study designed to assess the relationship between plasma risperidone and 9-hydroxyrisperidone concentrations and \textit{CYP2D6} genotype [87]. No other psychotropic medication, except benzodiazepines as sedative/hypnotic, was given to the patients. Blood samples for the determination of risperidone and 9-hydroxyrisperidone concentrations were obtained between 8:00 and 9:00 a.m., approximately 12 hours after the bedtime dose, after a minimum of 4 weeks of risperidone therapy at a stable dose.
Analytical methods

Genotyping
Genomic DNA was isolated from peripheral leukocytes with Qiagen Blood and Cell Culture kit (Qiagen, Hilden, Germany) according to the guidelines by the manufacturer.

**DRD2, DRD3, HTR2A, and HTR2C genotyping**
Polymerase chain reaction (PCR) followed by digestion with restriction enzymes (RFLP) according to the methods previously described were used to investigate the Taq1A [145], Ser311Cys [146] and -141C Ins/Del [112] polymorphisms of DRD2, the Ser9Gly [147] polymorphism of DRD3 gene, the 102T/C [148] and His452Tyr [127] polymorphisms of HTR2A gene, the -997G/A, -759C/T, -697G/C [131] and Cys23Ser [149] polymorphisms of HTR2C gene, with minor modifications.

**CYP1A2 genotyping**
The -3860G/A [73], the -2467delT [73], the -163C/A [74], the -739T/G and -729C/T [76] polymorphisms of the CYP1A2 gene were analyzed with previously described PCR-RFLP methods.

**CYP2D6 genotyping**
The CYP2D6*3 and CYP2D6*4 alleles were identified by allele-specific PCR followed by digestion with restriction enzymes [150]. The CYP2D6*5 allele was investigated by long-PCR analysis, and the CYP2D6*6 allele by tetra-primer PCR [151]. Alleles with neither CYP2D6*3, *4 nor *6 specific mutations, nor identified as CYP2D6*5, were classified as functional alleles. All samples were further analyzed by long-PCR for the duplicated/ multi-duplicated CYP2D6 gene [152, 153]. In study V, the CYP2D6 genotype was available for most of the patients from the previous study [87]. The newly included patients were genotyped with the methods previously described.

**CYP3A4/5 and ABCB1 genotyping**
The CYP3A4*1B [90], CYP3A4*3 [91], CYP3A4*4 [92], CYP3A5*2 [95] and CYP3A5*6 [95] alleles were identified by PCR-RFLP, while the presence of the CYP3A5*3 allele was investigated by TaqMan™ allelic discrimination in the ABI PRISM 7000 Sequence Detection System [154]. The ABCB1 polymorphisms were analyzed with real-time PCR by TaqMan kits purchased from Applied Biosystems according to the guidelines of the manufacturer.
Drug analyses

Analysis of perphenazine plasma concentrations (Study I)
Plasma perphenazine concentrations were determined at the Department of Clinical Pharmacology, Huddinge University Hospital, Stockholm, Sweden, by HPLC, according to a previously published method [155]. The limit of quantification was 0.1 nmol/L.

Analysis of clozapine and N-desmethyclozapine serum concentrations (Study III, IV)
Serum concentrations of clozapine and its N-desmethyl metabolite were analyzed at the section of Clinical Pharmacology, Akademiska Laboratoriet, Uppsala University Hospital, by HPLC. In brief, serum was alkalized and clozapine and its metabolite were extracted with hexan-dichlor-methan. Propyl clozapine was used as internal standard. The organic layer was evaporated to dryness and the residue was dissolved in 50 % methanol solution. The sample was then injected into an HPLC system [column Lichrosphere 60 RP-Select B, 75 x 4.6 mm (5 μm), Merck, Darmstadt, Germany] with a UV detector set to 240 nm. The limit of quantification was 150 nmol/L for both clozapine and N-desmethyclozapine.

Analysis of olanzapine and N-desmethylolanzapine serum concentrations (Study IV)
The serum concentrations of olanzapine and N-desmethylolanzapine were determined in samples taken 12-14 hours after drug intake by HPLC at the Department of Clinical Chemistry and Pharmacology, Lund University Hospital. The limit of quantification was 5 nmol/L for both olanzapine and N-desmethylolanzapine [58].

Analysis of risperidone and 9-hydroxyrisperidone plasma concentrations (Study V)
Plasma concentrations of risperidone and 9-hydroxyrisperidone were measured in samples taken 12 hours after drug intake in duplicate by HPLC, as previously described [31, 156]. The interday coefficient of variation was less than 8.2% for risperidone and less than 6.5% for 9-hydroxyrisperidone and the limit of quantification was 2 nmol/L for both analytes.

Metabolic parameters

Determination of blood glucose, hormones, lipids and HOMA index for insulin resistance (Study III and IV)
Blood glucose levels were determined by a glucose-oxidase method using the 950 Immunologic-Rate-Colorimetric system (Johnson and Johnson Clinical Diagnostics, Inc., N.Y.). Insulin and C-peptide were measured with
fluoroimmunometric assays using commercial kits (Delfia insulin and Delfia C-peptide, Wallac, Inc., Turku, Finland). Triglyceride and cholesterol concentrations were determined by enzymatic methods [157, 158]. The Homeostasis Model Assessment index for insulin resistance (HOMA-IR) was calculated according to the formula: fasting insulin concentration (μU/mL) x fasting glucose concentration (mmol/L) / 22.5 [159, 160].

Statistics

Kruskal-Wallis and Mann-Whitney tests were used in order to compare the group characteristics. In addition, the strength of the linear relationship between two parameters was calculated using the Spearman rank correlation coefficient ($r_s$) (Prism 4 Software, GraphPad Inc., San Diego, California, USA). Fisher’s exact test and Chi square test were used for the comparisons of allele or haplotype carrier frequencies in patient groups. Haplotype and multiple model analyses were performed using Statistical Analyses Software (SAS), version 9.1. P-values below 0.05 were accepted as statistically significant.
Results

Linkage disequilibrium analyses and haplotype frequencies

HTR2A (Study I, IV)
There was complete linkage disequilibrium (LD) between -1438A/G and 102T/C polymorphisms in Swedish patients. Moreover, pairwise LD patterns between 102T/C and 452Tyr polymorphisms were similar in Estonian and Swedish patients. A few haplotypes were identified.

Table 1. Pairwise linkage disequilibrium analyses (r^2) between HTR2A polymorphisms and haplotype frequencies

<table>
<thead>
<tr>
<th></th>
<th>Estonian patients (Study I)</th>
<th>Swedish patients (Study IV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>102T/C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-1438A/G</td>
<td></td>
<td></td>
</tr>
<tr>
<td>102T/C</td>
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<td>1.0</td>
</tr>
<tr>
<td>His452Tyr</td>
<td></td>
<td>0.103</td>
</tr>
<tr>
<td>102T/C</td>
<td></td>
<td>0.103</td>
</tr>
<tr>
<td>His452Tyr</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

HTR2A Haplotype | -1438A/G | 102T/C | His452Tyr | Frequency | Frequency |
<table>
<thead>
<tr>
<th></th>
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<td></td>
<td></td>
<td></td>
<td>Estonian patients</td>
<td>Swedish patients</td>
</tr>
<tr>
<td>1</td>
<td>G</td>
<td>C</td>
<td>His</td>
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<td>0.64</td>
</tr>
<tr>
<td>2</td>
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<td>T</td>
<td>His</td>
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<td>3</td>
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</tr>
<tr>
<td>4</td>
<td>G</td>
<td>C</td>
<td>Tyr</td>
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<td>-</td>
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</table>

*The -1438A/G was not evaluated in Estonian patients. However, there is complete linkage disequilibrium (LD) between -1438A/G and 102T/C polymorphisms in Swedish patients and also reported in other populations.
**HTR2C (Study I, II, IV)**

The LD patterns and haplotype frequencies were very similar in Estonian and Swedish patients and slightly different in the Italian population. There was complete linkage disequilibrium ($r^2=1$) between -995G/A and -759T/C polymorphisms in Italian subjects.

*Table 2.* Pairwise LD analyses ($r^2$) between HTR2C polymorphisms and haplotype frequencies

**Estonian patients (Study I)**

<table>
<thead>
<tr>
<th>-759C/T</th>
<th>-759C/T</th>
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<tbody>
<tr>
<td>-697G/C</td>
<td>0.494</td>
</tr>
<tr>
<td>Cys23Ser</td>
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</table>

**Swedish patients (Study IV)**

<table>
<thead>
<tr>
<th>-759C/T</th>
<th>-759C/T</th>
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</thead>
<tbody>
<tr>
<td>-697G/C</td>
<td>0.468</td>
</tr>
<tr>
<td>Cys23Ser</td>
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</table>

**Italian patients and healthy volunteers (Study II)**

<table>
<thead>
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<th>-997G/A</th>
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</thead>
<tbody>
<tr>
<td>-759C/T</td>
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<tr>
<td>-697G/C</td>
<td>0.386</td>
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<tr>
<td>Cys23Ser</td>
<td>0.044</td>
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</table>

**Frequency**

<table>
<thead>
<tr>
<th>HTR2C Haplotypes</th>
<th>-997G/A</th>
<th>-759C/T</th>
<th>-697G/C</th>
<th>Cys23Ser</th>
<th>Estonian patients</th>
<th>Swedish patients</th>
<th>Italian patients</th>
<th>Healthy volunteers</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>G</td>
<td>C</td>
<td>G</td>
<td>Cys</td>
<td>0.68</td>
<td>0.66</td>
<td>0.58</td>
<td>0.67</td>
</tr>
<tr>
<td>B</td>
<td>A</td>
<td>T</td>
<td>C</td>
<td>Cys</td>
<td>0.18</td>
<td>0.19</td>
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<td>0.18</td>
</tr>
<tr>
<td>C</td>
<td>G</td>
<td>C</td>
<td>C</td>
<td>Ser</td>
<td>0.10</td>
<td>0.10</td>
<td>0.18</td>
<td>0.12</td>
</tr>
<tr>
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<td>G</td>
<td>C</td>
<td>C</td>
<td>Cys</td>
<td>0.03</td>
<td>0.04</td>
<td>0.05</td>
<td>0.02</td>
</tr>
<tr>
<td>E</td>
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<td>Ser</td>
<td>0.01</td>
<td>0.01</td>
<td>0.02</td>
<td>0.01</td>
</tr>
</tbody>
</table>

* The -997G/A is in complete linkage disequilibrium with -759C/T and was only analyzed in the Italian population.
**ABCB1 (Study V)**

There was close linkage disequilibrium between *ABCB1* polymorphisms 1236C/T, 2677G/T/A and 3435C/T.

*Table 3. Pairwise LD analyses (r²) between *ABCB1* polymorphisms and haplotype frequencies*

<table>
<thead>
<tr>
<th></th>
<th>1236C/T</th>
<th>1236C/T</th>
<th>2677G/T</th>
<th>2677G/T</th>
<th>3435C/T</th>
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<tbody>
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<tr>
<td>2677G/T</td>
<td>0.679</td>
<td></td>
<td></td>
<td>0.643</td>
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<tr>
<td>3435C/T</td>
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<td></td>
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</table>

**Genotype-phenotype relationships**

**Therapeutic response to short term perphenazine treatment**

Thirty-seven (80%) of the perphenazine treated patients were classified as responders, while ten were nonresponders. The variant allele frequencies of neither dopamine nor serotonin receptor genes differed between responders and nonresponders.

**Extrapyramidal side effects**

Twenty-five patients (54%) experienced EPS during perphenazine treatment. The frequencies of *DRD2 Taq1A1, 311Cys, -141CDel* and *DRD3 9Gly* alleles did not differ between patients with and without EPS.

On the other hand, patients with EPS had significantly higher frequency of the 102C allele of *HTR2A* (p=0.02, OR 3.18, 95% CI 1.153-8.75) and the -697C (p=0.01, OR 4.30, 95% CI 1.42-13.0) and 23Ser alleles (p=0.02) of *HTR2C*, compared to patients without EPS (Figure 5). In multiple model analyses, including variant allele carriage, age, gender and the duration of antipsychotic treatment as co-variants, the differences in the frequencies of
the HTR2A 102C and HTR2C -697C alleles remained significant between patients with and without EPS (p=0.03, p=0.01, respectively).

Figure 5. Comparison of HTR2A and HTR2C variant allele frequencies between patients with EPS and without EPS (Study I)

In the second study, 51 patients were classified as having EPS, while 48 patients did not have either current symptoms or history of movement disorders. Of the 51 patients who had current or documented history of EPS, 22 had Parkinsonism, 13 dystonia, 6 tardive dyskinesia, 5 Parkinsonism and dystonia, and 5 Parkinsonism and tardive dyskinesia. The mean antipsychotic daily dose, expressed in chlorpromazine equivalents, did not differ between patients with and without EPS. Among the patients who experienced EPS, 29 received anticholinergic drugs (biperiden or orphenadrine).

The association between the HTR2C 23Ser allele and the risk to develop EPS was also observed in this study population. The frequency of 23Ser allele was higher among patients with EPS (0.29) compared to patients without EPS (0.15) and healthy volunteers (0.13) (p=0.025, Figure 6). Comparison of the 23Ser allele frequency in patients with and without EPS gave an OR of 2.4 with 95% CI 0.90–6.7 (p=0.076). In multiple model analyses, including the 23Ser allele frequency, CYP2D6 genotype, age and chlorpromazine equivalent dose as co-variants, the 23Ser allele frequency was the closest to significance (p=0.09) compared to other co-variants and an OR of
2.5 (95% CI 0.9-7.1). No significant influence of CYP2D6 genotype, age and chlorpromazine equivalent dose was observed (p-values 0.21-0.97)

*Figure 6. Comparison of HTR2C variant allele frequencies between patients with EPS and without EPS (Study II)*

Furthermore, a statistically significant difference (p=0.04) was found in the frequency of a HTR2C haplotype including the -997G,-759C, -697C and 23Ser alleles between patients with EPS (0.26) compared to patients without EPS (0.10) and healthy volunteers (0.12) in study II. When comparing the frequency of this haplotype between patients with and without EPS, a borderline significance (p=0.05 with an OR of 2.9 (95% CI 0.96-9.01)) was observed. Multiple model analyses, including this haplotype, CYP2D6 genotype, age and chlorpromazine equivalent dose as co-variants, identified the haplotype as the closest to statistical significance (p=0.057, OR 3.2 (95% CI 0.97-10.4)).

Evaluation of male patients from study I together with patients from study II showed that this haplotype is significantly more frequent among male patients with EPS (0.24) compared to patients without EPS (0.09) (p=0.025, OR 3.3, 95% CI 1.11-9.57).

**Steady state serum clozapine and N-desmethylclozapine levels**

Smoking status had no influence on dose corrected clozapine and N-desmethylclozapine concentrations (C/D) or clozapine / N-desmethyl-
clozapine ratios. Patients carrying two of the CYP1A2 -3860A, -2467delT, -163C, -739G or -729T alleles had higher clozapine and N-desmethylclozapine C/Ds compared to patients who carried one or none of these alleles (p< 0.05, Figure 7).

The clozapine / N-desmethylclozapine ratio was not influenced by CYP1A2 genotype. The CYP2D6 genotype did not correlate with clozapine C/D, N-desmethylclozapine C/D or clozapine / N-desmethylclozapine ratio in this study population.

![Figure 7. Comparison of clozapine and N-desmethylclozapine plasma levels in patients carrying none, one or two of the CYP1A2 -3860A, -2467delT, -163C, -739G or -729T alleles.](image)

Metabolic adverse effects induced by clozapine and olanzapine

In study III, the only positive finding with respect to the genotypes evaluated was that the -3860A and -2467delT CYP1A2 variants were more frequent in patients with elevated insulin levels compared to patients with normal insulin levels (p=0.04).

In study IV, olanzapine-treated patients who carried haplotype C (-759C, -697C, 23Ser) had higher body mass index (BMI) compared to patients who carried haplotype B (-759T, -697C, 23Ser) (p=0.029). Haplotype C carriers also had higher C-peptide levels compared to haplotype B (p=0.029) carriers (Figure 8). No such associations were found in the clozapine group or in the overall study population. In the olanzapine group, patients who carried the haplotype 2 (-1438A, 102T, 452His) for HTR2A had higher C-peptide levels compared to patients with haplotype 3 (-1438A, 102T, 452Tyr) (p=0.034). This association was significant in the overall study population (p=0.019) but not in the clozapine group (p>0.05).
Among clozapine-treated patients the frequency of HTR2C haplotype A (-759C, -697G, 23Cys) carriers was significantly higher in patients with obesity (BMI ≥ 30 kg/m²) (7/8, 87.5%) compared to patients with lower BMI (2/10, 20%) (p=0.015, OR 28, 95% CI 2-380). On the other hand, the frequency of HTR2C haplotype B carriers tended to be lower among patients with obesity (1/8 12.5%) compared to patients without obesity (6/10 60%) (p=0.065, odds ratio 11, 95% confidence interval 0.91-122, Figure 9). These associations were not observed in the olanzapine group or in the overall study population. No association was found between HTR2A haplotypes and obesity.
Steady state plasma levels of risperidone, 9-hydroxyrisperidone and active moiety

The C/Ds of risperidone showed 68-fold (0.3-22 nmol/L/mg, median 3 nmol/L/mg), 9-hydroxyrisperidone 10-fold (6-64 nmol/L/mg, 27 nmol/L/mg) and active moiety 10-fold (7-70 nmol/L/mg, 32 nmol/L/mg) variation.

The CYP3A5 genotype did not influence C/Ds of risperidone, 9-hydroxyrisperidone, or active moiety, or the risperidone/9-hydroxyrisperidone ratio (p>0.05). In accordance with the previous study [87], the CYP2D6 genotype associated significantly with risperidone C/D (p=0.008), but not with 9-hydroxyrisperidone or active moiety C/D (p>0.05).

Patients homozygous for the ABCB1 1236T/2677T/3435T haplotype (n=11) had significantly lower C/Ds of 9-hydroxyrisperidone (*p=0.026) and active moiety (**p=0.028) than patients with other genotypes, while no significant influence of this haplotype was observed on risperidone C/Ds or risperidone/9-hydroxyrisperidone ratio (Figure 10).

Figure 10. Comparison of 9-hydroxyrisperidone and active moiety levels in patients homozygous for ABCB1 1236T/2677T/3435T haplotype with other genotypes
In multiple model analysis, including *CYP2D6* genotype and *ABCB1* haplotypes as co-variants, the influence of the *CYP2D6* genotype on risperidone C/Ds remained highly significant (p=0.0002), while the effect of *ABCB1* haplotype on 9-hydroxyrisperidone (p=0.049) and active moiety (p=0.062) C/Ds was marginal. *CYP2D6* and *ABCB1* genotypes explained 34% of the variation in plasma risperidone C/D, 13% in the 9-hydroxyrisperidone C/D and 16% in the active moiety C/D.
We observed several associations between adverse effects of antipsychotics and polymorphisms in genes involved in both pharmacokinetics and pharmacodynamics of these drugs.

Pharmacogenetic studies have long been focused on the polymorphisms in genes influencing the plasma levels of antipsychotic drugs as a source of interindividual variability in clinical outcome. However, so far this approach has not provided enough evidence to support the wide use of pharmacogenetic testing as a tool for dose individualization during antipsychotic treatment. More recently, an increasing number of studies have evaluated the impact of polymorphisms in genes involved in pharmacodynamics of antipsychotic drugs on the variability in their clinical outcome. The findings provided by this approach have been difficult to interpret since most of the antipsychotic drugs influence multiple receptor types.

Among drug target gene polymorphisms, associations have been reported between the DRD2 Taq1A1 and the DRD3 9Gly variants and an increased risk for EPS [60, 121, 122, 161]. These associations, however, were not supported by our findings in patients receiving perphenazine monotherapy. Differences in study design, sample size, definition and assessment of the therapeutic and adverse effects, and patient characteristics are factors that might have led to these discrepancies.

The functional consequences of polymorphisms in serotonin receptor coding genes have not been clearly identified yet. However, some evidence in the literature points towards possible alterations in receptor expression and function. The positive finding concerning the HTR2A 102C allele and the increased risk of EPS in our study in perphenazine treated patients is in line with previously reported association between this allele and tardive dyskinesia in a meta-analysis that included 635 patients with different ethnic backgrounds [61]. The HTR2A 102T/C is a silent polymorphism in close linkage disequilibrium with the promoter polymorphism -1438A/G. The -1438G variant has been reported to be associated with reduced promoter activity and lower expression of the receptor protein compared to the -1438A variant [125]. The lower EPS incidence of atypical antipsychotics has been attributed to their high 5-HT2A receptor antagonistic affinity. Thus, an increased 5-HT2A receptor activity might theoretically be expected to cause an increased EPS risk in patients carrying the 102C and -1438G alleles.
The *HTR2C* -697C and 23Ser alleles were also found to be associated with increased EPS risk in patients receiving perphenazine monotherapy. Interestingly, the association between the *HTR2C* -697C allele and EPS found in this study was not confirmed in a larger population consisting of only male patients in the second study. The *HTR2C* gene is located on the X chromosome (females carrying two alleles and males one). It is possible that the allele frequency calculations in different studies might have been influenced by gender distribution. On the other hand, the -697C allele was present together with the -997G, -759C, and 23Ser alleles in *HTR2C* haplotype C that was found to be more frequent among patients with EPS compared to patients without EPS and healthy volunteers in the second study. Previously, the -697C allele has been shown to be associated with tardive dyskinesia in Chinese male chronic schizophrenic patients [139]. Likewise, Segman et al. [63] reported a significant association between the 23Ser allele and orofacial dyskinesia. It is possible that the associations found in our first study and in the study of Zhang et al. [139] concerning the -697C allele could be related to the *HTR2C* haplotype C.

The association between the 23Ser allele and the risk to develop EPS in study I was also observed in the larger male population of study II. Additionally, in Study II, a trend towards an association (p=0.057, OR 3.2; 95% CI 0.97-10.4) between the *HTR2C* haplotype C (including 23Ser allele together with the -997G,-759C and -697C alleles) with the occurrence of EPS was found in multiple model analyses, when this haplotype, *CYP2D6* genotype, age and chlorpromazine equivalent dose were evaluated as co-variants in the second study. Furthermore, evaluation of male patients from study I together with patients from study II showed that this haplotype is significantly more frequent among patients with EPS (0.24) compared to patients without EPS (0.09) (p=0.025, OR 3.3, 95% CI 1.11-9.57). 5-HT2C receptors have been reported to exert an inhibitory control on dopaminergic transmission in basal ganglia [47]. However, the functional effects of *HTR2C* polymorphisms on receptor expression and activity have been controversial. Haplotypes including the -697C and -759T alleles were shown to have significantly lower promoter activities compared to haplotypes with the -697G and -759C alleles *in vitro* [132]. Moreover, the Cys23Ser polymorphism has been reported to alter the constitutive activity of the receptor with the 23Ser allele leading to a higher constitutive activity *in vitro* compared to the 23Cys variant [134]. Thus, a high 5-HT2C activity is expected in patients carrying the *HTR2C* -759C, -697G and 23Ser alleles. An increased activity of the 5-HT2C receptor may lead to an increased inhibitory control on dopaminergic neurons in the striatum and a higher risk of EPS.

The *HTR2C* and *HTR2A* gene polymorphisms also showed significant associations with metabolic adverse effects of olanzapine and clozapine. Previously, patients carrying the *HTR2C* -759T allele have been reported to gain less weight compared to -759C carriers during atypical antipsychotic treat-
ment [62, 141, 142]. This association was confirmed in a meta-analysis including 588 patients (mainly clozapine treated) from 10 studies [140]. Moreover, a haplotype including the -697C and -759T alleles was associated with leanness and absence of diabetes, while another haplotype including the -697G and -759C alleles associated with obesity [131]. However, the 23Ser variant was not evaluated in these haplotypes. In our study, the \textit{HTR2C} haplotype C including the -759C, -697C and 23Ser alleles was found to be associated with higher BMI and C-peptide levels in olanzapine-treated patients while the \textit{HTR2C} haplotype A (-759C, -697G, 23Cys) was associated with obesity in clozapine treated patients. Both haplotypes included the -759C allele, pointing towards the same direction as previous studies, i.e. an increased susceptibility of patients who carry this allele for higher BMI and C-peptide levels. The linkage disequilibrium between -697G/C and -759C/T polymorphisms might have caused the inconsistent findings concerning the -697G/C polymorphism.

Polymorphisms of \textit{HTR2A} have been evaluated only in a limited number of studies in relation to antipsychotic induced metabolic changes. In one study an association between the 102C/C genotype and greater weight gain during 6 weeks of risperidone treatment was shown [162], while in another, no effect of 102T/C or His452Tyr polymorphisms on weight gain was seen in patients treated with clozapine for 6 weeks [65]. In our study, the \textit{HTR2A} haplotype 3 (-1438A, 102T, 452Tyr) was associated with lower C-peptide levels in patients treated with olanzapine or clozapine. The 452Tyr allele has been shown to be associated with decreased serotonin-induced calcium mobilization and reduced activation of phospholipases C and D \textit{in vitro} [127, 163]. Thus, a decreased activity of 5-HT\textsubscript{2A} receptor in haplotype 3 carriers might have led to lower C-peptide levels.

The frequencies of the \textit{HTR2C} variant alleles analyzed did not differ significantly between the Estonian, Italian and Swedish populations studied. The allele frequencies, LD patterns and haplotype frequencies were very similar in Estonian and Swedish patients, and slightly different in Italian patients in terms of higher 23Ser allele and \textit{HTR2C} haplotype C frequencies. Moreover, no significant differences between Italian patients and healthy volunteers were observed for any of the allele frequencies. Nevertheless, we obtained similar results in Estonian and Italian patients concerning the association of 23Ser allele with the risk of developing EPS. Furthermore, the combined analysis of male Estonian patients from study I and male Italian patients from study II resulted in a 3 fold increased risk for the occurrence of EPS in \textit{HTR2C} haplotype C carriers.

Genetic variants of the enzymes catalyzing the metabolism of antipsychotic drugs potentially influence both therapeutic and adverse effects of these drugs. We observed a significant correlation between the \textit{CYP1A2} -3860A, -2467delT, -163C, -739G and -729T alleles and serum clozapine and N-desmethyleclozapine C/Ds, while no impact of \textit{CYP2D6} genotype was obser-
Our findings support previous reports, suggesting an impact of CYP1A2 polymorphisms on serum clozapine and N-desmethylclozapine concentrations [77, 78]. Moreover, the frequency of CYP1A2 polymorphisms located in the 5'-flanking region of the gene (-3860A, -2467delT) was higher among patients with elevated fasting insulin levels compared to patients with normal insulin levels. Thus, additional to their influence on serum drug concentrations, a link between CYP1A2 polymorphisms and metabolic adverse effects of clozapine treatment has been observed.

Previously, the presence of ABCB1 2677T and 3435T alleles has been shown to reduce expression and/or activity of P-gp [106, 164, 165], which might lead to an increased intestinal absorption and central nervous system and CNS penetration or a decreased excretion and consequently, to increased blood levels of P-gp substrates. However, we have observed the opposite as patients homozygous for the ABCB1 haplotype 1236T/2677T/3435T had significantly lower 9-hydroxyrisperidone and active moiety C/Ds than patients with other genotypes. As both risperidone and 9-hydroxyrisperidone are highly lipophilic agents, it is possible that a higher central nervous system concentration could occur due to a decreased efflux of the compounds at the blood brain barrier in ABCB1 haplotype 1236T/2677T/3435T carriers. However, we do not have clinical outcome data to support this theory.

The interindividual variation of risperidone C/D was 68-fold in our study population, while 9-hydroxyrisperidone C/D varied 10-fold and active moiety C/D 10-fold. The multiple model analyses showed that CYP2D6 and ABCB1 genotypes could explain 34% of the variation in plasma risperidone C/D, 13% of the variation in 9-hydroxyrisperidone C/D and 16% of the active moiety C/D variation. 9-hydroxyrisperidone has recently been marketed as a new antipsychotic drug (paliperidone) with the expectations of reduced interindividual variability in plasma levels [166]. Although only a relatively small portion of the variation in 9-hydroxyrisperidone steady state plasma levels could be explained by ABCB1 genotype in our study, one could speculate that patients carrying different ABCB1 genotypes prescribed paliperidone may experience variations in plasma levels that may potentially influence therapeutic as well as adverse effects of the treatment.

A general limitation of our studies was the small number of patients evaluated. Therefore, the associations found in our studies should be confirmed in larger populations with a prospective study design in order to determine their clinical importance and applicability for pharmacogenetic testing.
To identify the risk factors for the occurrence of adverse effects induced by antipsychotic drugs is of pivotal clinical importance due to their high prevalence, influence on compliance and impact on patient morbidity and mortality, given the need for long term treatment in schizophrenia. Polymorphisms of \textit{HTR2A, HTR2C, CYP1A2, CYP2D6} and \textit{ABCB1} genes may potentially influence the clinical outcome of antipsychotic therapy with classical and atypical antipsychotics.

EPS in patients on short term perphenazine is more likely to occur in \textit{HTR2A} 102C allele or \textit{HTR2C} -697C and 23Ser allele carriers. Also, patients treated with classical antipsychotics carrying the \textit{HTR2C} 23Ser allele or the \textit{HTR2C} haplotype C including the -759C, -697C and 23Ser alleles might be at higher risk for EPS.

The \textit{CYP1A2}, but not \textit{CYP2D6} polymorphisms influence clozapine and N-desmethylclozapine serum concentrations. Clozapine treated patients carrying the \textit{CYP1A2} -3860A, -2467delT, -163C, -739G or -729T alleles are exposed to higher clozapine and N-desmethylclozapine serum concentrations than subjects with other genotypes, and might have an increased risk for metabolic adverse effects.

Both \textit{HTR2C} and \textit{HTR2A} polymorphisms associate with metabolic abnormalities in olanzapine treated patients. \textit{HTR2C} haplotype C also correlates with higher BMI and C-peptide levels in olanzapine treated patients. On the other hand, \textit{HTR2A} haplotype 3 associates with lower levels of C-peptide in patients treated with olanzapine and clozapine.

\textit{ABCB1} polymorphisms contribute to the interindividual variation in steady state plasma levels of 9-hydroxyrisperidone and the active moiety. Further studies are needed to evaluate the clinical implication of this effect.
Future Perspectives

Prospective clinical pharmacogenetic studies with careful phenotype assessment allowing evaluation of relevant co-variants together with the genotypes of interest are needed to define the predictive value of genetic testing prior to treatment initiation. Such information could be used for prevention of adverse affects, dose titration and choice of optimal drug.

Genotyping for \textit{HTR2A}, \textit{HTR2C}, \textit{CYP1A2}, \textit{CYP2D6} and \textit{ABCB1} polymorphisms may potentially provide useful information to identify patients at higher risk to develop EPS or metabolic adverse effects during treatment with antipsychotic drugs.

Future studies preferring haplotype approaches instead of single SNP based comparisons might help to clarify earlier reported inconsistent genotype-phenotype associations. Moreover, increasingly available SNP, LD and haplotype databases will also contribute to the identification of clinically important genotype-phenotype relationships. Genotyping technologies improve rapidly and are becoming easily available for researchers as well as for clinicians. It should soon be possible to utilize the pharmacogenetic knowledge to improve the clinical outcome of antipsychotic treatment.
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A doctoral dissertation from the Faculty of Medicine, Uppsala University, is usually a summary of a number of papers. A few copies of the complete dissertation are kept at major Swedish research libraries, while the summary alone is distributed internationally through the series Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine. (Prior to January, 2005, the series was published under the title “Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine”.)