ORIGINAL ARTICLE

TITLE

Impact of *CYP2D6* on serum concentrations of flupentixol, haloperidol, perphenazine and zuclopenthixol

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Keywords

Antipsychotics, serum concentrations, therapeutic drug monitoring, CYP450, genetic polymorphism

Running head

Impact of *CYP2D6* on antipsychotic levels

WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- *CYP2D6* is believed to be involved in the metabolism of flupentixol, haloperidol, perphenazine and zuclopenthixol.
- The *CYP2D6**41 allele is classified as a moderately reduced function allele.

WHAT THIS STUDY ADDS

- The present study revealed significantly higher dose-adjusted serum concentrations in perphenazine and zuclopenthixol *CYP2D6* poor metabolizers and intermediate metabolizers compared to normal metabolizers.
- *CYP2D6* is of minor or no importance to haloperidol and flupentixol metabolism.
- The *CYP2D6**41 allele appears to have a reduced function close to non-functional variant alleles.

ABSTRACT

AIMS

To investigate the impact of *cytochrome P450 2D6 (CYP2D6)* on dose-adjusted serum concentrations of flupentixol, haloperidol, perphenazine and zuclopenthixol in a therapeutic drug monitoring (TDM) cohort of psychiatric patients. We also especially studied the functional impact of *CYP2D6*41* on dose-adjusted serum concentrations in the perphenazine-treated patients.

METHODS

Serum concentrations of flupentixol, haloperidol, perphenazine and zuclopenthixol from *CYP*-genotyped patients were extracted retrospectively from a routine TDM database in the period March 2005 to May 2019. Samples were divided into three *CYP2D6* phenotype subgroups according to genotype; normal metabolizers (NMs), intermediate metabolizers (IMs) and poor metabolizers (PMs). The effect of *CYP2D6* phenotype on dose-adjusted serum concentrations of the four antipsychotics was evaluated by multivariable mixed model analyses.

RESULTS

Mean dose-adjusted serum concentrations of perphenazine (564 samples) were 3.9-fold and 1.6-fold higher in *CYP2D6* PMs and IMs, respectively, compared with NMs ($p<0.001$ and $p<0.01$). For zuclopenthixol (658 samples), mean dose-adjusted serum concentrations were about 1.5-fold and 1.3-fold higher in *CYP2D6* PMs and IMs, respectively, compared with NMs ($p<0.01$ and $p<0.001$). *CYP2D6* was of minor or no importance to haloperidol (320 samples) and flupentixol (115 samples). In our data material, the genotype *CYP2D6 *1/*41* appears to have a similar impact on dose-adjusted serum concentrations of perphenazine as **1/null* (*null* = variant allele encoding no enzyme function).

CONCLUSIONS

This study shows that *CYP2D6* is important for the metabolism of perphenazine and zuclopenthixol, but not for haloperidol and flupentixol. The *CYP2D6*41* allele appears to have a reduced function close to non-functional variant alleles.

INTRODUCTION

Even though newer antipsychotic agents are increasingly prescribed among antipsychotic medicines, there are still a lot of patients using the old antipsychotics [flupentixol](#), [haloperidol](#), [perphenazine](#) and [zuclopenthixol](#). The metabolism of the last three is in standard sources of drug information described as being substrates of both cytochrome P450 2D6 ([CYP2D6](#)) and [CYP3A4](#) [1-5]. The Arbeitsgemeinschaft für Neuropsychopharmakologie und Pharmakopsychiatrie (AGNP) therapeutic drug monitoring (TDM) expert group states CYP2D6 to be the main CYP enzyme involved in the metabolism of all of these four antipsychotics [6]. [CYP1A2](#) is also possibly involved in the metabolism of haloperidol and perphenazine [2, 3, 6].

The CYP2D6 enzyme is thought to be involved in the metabolism of up to 25% of the medicines used today. *CYP2D6* is highly polymorphic, and more than 90 allelic variants are described, which may cause absent, reduced, unchanged or increased enzyme activity [7]. Patients are divided into four different CYP2D6 metabolizer groups depending on their ability to metabolize CYP2D6 substrates, *i.e.* poor metabolizers (PMs), intermediate metabolizers (IMs), normal metabolizers (NMs), and ultrarapid metabolizers (UMs) [7]. There has been some disagreement in the field regarding how certain genotypes should be translated into phenotype metabolizing group. In this paper we decided to use the last consensus recommendation from the Clinical Pharmacogenetics Implementation Consortium (CPIC) and Dutch Pharmacogenetics Working Group (DPWG) for the CYP2D6 genotype to phenotype translation, published in January 2020 [8].

The extent to which different CYP2D6 phenotypes affect the serum concentration of these four old antipsychotics is sparingly described. Most is known for zuclopenthixol and perphenazine. In an early publication by Jerling et al., patients treated with zuclopenthixol (n=20) and perphenazine (n=16) were genotyped for *CYP2D6* (only genotyped for the variant alleles *3 and *4). They found that NMs had a 2-fold and 3-fold higher clearance of zuclopenthixol and perphenazine, respectively, compared to PMs.[9] A Danish study from the same year found similar results for zuclopenthixol in 108 *CYP2D6* genotyped patients (*3 and *4) [10]. In a smaller study, 11 patients treated with haloperidol and 6 patients on zuclopenthixol were *CYP* genotyped [11]. Probably because of the small sample size, there were no significant differences in dose-adjusted serum concentrations between the CYP2D6 phenotype subgroups for the two drugs. Regarding flupentixol metabolism, there are no publications concerning the impact of CYP2D6 enzyme function.

Small studies and few included variant alleles, especially in the old publications, make the findings difficult to implement. In addition, the patient age, smoking habits, and concomitant medications can affect the results. At our laboratory, we now have TDM data from a large number of

CYP2D6 genotyped patients using flupentixol, haloperidol, perphenazine and zuclopenthixol, including information about concomitant medication, smoking habits and age. The aim of this study was to investigate to what extent genetic variation in *CYP2D6* affects the dose-adjusted serum concentrations of these four old antipsychotic medicines in psychiatric patients. In addition, we analysed the impact of the patient's age and the effect of smoking on the dose-adjusted serum concentrations. Because the classification of *CYP2D6**41 as a reduced function variant allele is debated, we also especially studied the functional impact of *CYP2D6**41 on the dose-adjusted serum concentrations in the perphenazine-treated patients.

METHODS

Patients and samples

The routine TDM database at the Center for Psychopharmacology, Diakonhjemmet Hospital, Oslo, Norway, contains a large amount of serum concentration measurements of antipsychotic medicines, as well as information on genotype for many of the same patients. Serum concentrations of flupentixol, haloperidol, perphenazine and zuclopenthixol were extracted retrospectively from this database from March 2005 to May 2019. All the patients with one or several measurements of the above mentioned antipsychotics, and with known *CYP2D6* genotype, constituted the study population. For patients using more than one antipsychotic, all serum concentrations were included. For some patients *CYP* genotyping had been performed prior to initiation of antipsychotic treatment, whereas for others genotyping was done after initiation of antipsychotic treatment. All the *CYP* genotyping had been performed in the period November 2010 to May 2019.

Information about the determined serum concentrations, *CYP2D6* genotype, age, gender, daily drug dosage, and sampling time (time interval between last drug intake and sample withdrawal) were recorded from the TDM files. When available, information on smoking habits was also recorded. When multiple serum concentration analyses of an antipsychotic were available from the same patient, all measurements were considered for inclusion. This was possible due to the choice of statistical models (see later). Serum concentration measurements were included in the study if the TDM files provided information on tablet dosage only (patients receiving acute or long-acting injections were not included), serum sampling had been performed 10-26 hours after the last dose intake, and measured serum concentrations were within the lower and upper limits of quantification. The included serum concentrations sampled 10-26 hours after the last dose intake were assumed to be at steady-state, as this is regular practice by use of TDM.

Patients carrying multiplied, functional *CYP2D6* alleles were excluded from the study due to few samples. Further, in cases where the requisition forms provided information on possible noncompliance, or concomitant use of drugs inhibiting or inducing the respective *CYP* enzymes involved in flupentixol, haloperidol, perphenazine, and zuclopenthixol metabolism, measurements were also excluded from the study. Requisition forms were available for inspection for most of the included samples. However, for approximately 11% of the included samples, the requisition forms were unavailable. The “P450 Drug Interaction Table” published at the website <https://drug-interactions.medicine.iu.edu/> and the Norwegian drug interaction website www.legemiddelinteraksjoner.no were used as references to agents described as enzyme inhibitors/inducers [12, 13].

The study was approved by the Regional Committee for Medical and Health Research Ethics (ref. 2017/2191) and the Hospital Investigational Review Board.

Drug analyses

Serum analyses of flupentixol, haloperidol, perphenazine and zuclopenthixol were performed by validated and certified analytical methods developed for routine TDM analyses at the Center for Psychopharmacology, Diakonhjemmet Hospital, Norway. During the time span of the retrospective data collection, the analytical assays had been modified due to the renewal of the analytical instrumentation, but all modifications were cross-validated. Briefly, in the current ultra-performance liquid chromatography Orbitrap mass spectrometry with simultaneous full scan and tandem MS/MS (UPLC/HRMS) method, serum samples were purified by protein precipitation mixing 200 μ L of serum aliquot with 400 μ L of acetonitrile-methanol (90/10 vol/vol), which included internal standards, followed by centrifugation for 10 minutes (4000rpm at 4 °C). About 4 μ L of the purified serum samples were then injected into a Vanquish Binary UHPLC (Thermo Scientific, Waltham, MS, USA) coupled to a Q Exactive Orbitrap HRAM LC-MS/MS mass spectrometer (Thermo) with electrospray ionization operated in positive ionization mode. Chromatographic separation of all compounds was performed on a XBridge BEH C18 column (2.5 μ m, 2.1 $\times$ 75 mm; Waters). The mobile phase gradient was based on a mixture of acetonitrile and ammonium acetate buffer (pH 4.8) starting at 20% of organic solvent and increased to 95% within 2.1 minutes, resulting in a total run time of 4 minutes including reconditioning of the analytical column.

MS/MS detection was performed by multiple reactions monitoring at specific transitions for the different analytes and internal standards. Calibration curves (n=5) ranging from 0.5-30 nM for flupentixol, 1-40 nM for haloperidol, 0.5-15 nM for perphenazine, and 2-60 nM for zuclopenthixol fitted to linear polynomial functions gave $r^2 > 0.995$ for the different analytes. At both extremes of the calibration curves, the intra- and inter-day validation parameters of imprecision and inaccuracy showed less than 15% deviation (n=5) for all compounds. Flupentixol, haloperidol, perphenazine and zuclopenthixol concentrations were measured as nM.

CYP2D6 genotyping

The method for CYP2D6 genotyping included the identification of the non-functional variant alleles CYP2D6*3 (rs35742686), CYP2D6*4 (rs3892097), CYP2D6*5 (gene deletion), and CYP2D6*6 (rs5030655), the reduced function variant alleles CYP2D6*9 (rs5030656), CYP2D6*10 (rs1065852), and CYP2D6*41 (rs28371725), as well as allele multiplication. Analysis of CYP2D6 variant alleles were performed using TaqMan-based real-time PCR assays implemented for routine pharmacogenetic analyses at the Center for Psychopharmacology, Diakonhjemmet Hospital. Copy number analysis of

CYP2D6 (detecting either deletion or multiplication of *CYP2D6*) was performed with TaqMan Copy Number Assay targeting exon 9, with RNase P as an endogenous control. Absence of variant alleles was interpreted as presence of the functional wild-type allele (**1*).

Data analyses

Samples were divided into three *CYP2D6* phenotype subgroups according to genotype; normal metabolizers (NMs; including **1/*1*, **1/red*), intermediate metabolizers (IMs; including **1/null*, *red/red*, *null/red*), and poor metabolizers (PMs; including *null/null*) (*red* = variant allele encoding reduced enzyme function; *null* = variant allele encoding no enzyme function) (Table 1). To enable inclusion of multiple serum measurements from the same individuals, multivariable mixed model analyses were applied. In this type of multivariable analysis, the data is weighted according to the number of measurements per person, to account for the correlation in concentrations within each person. The overall effect of *CYP2D6* phenotype (using NMs as controls) on dose-adjusted serum concentrations (i.e. concentration to dose ratios; nmol/L/mg/day) and absolute serum concentrations of flupentixol, haloperidol, perphenazine and zuclopenthixol was evaluated. The differences in mean daily dosages between the *CYP2D6* phenotype subgroups for the four antipsychotics investigated were also examined. Additionally, for a subset of the data material, where information on smoking habits was available, the effect of cigarette smoke on dose-adjusted serum concentrations of the four antipsychotics was examined.

Furthermore, we assessed the specific significance of the different reduced function variant alleles, *CYP2D6*9*, **10*, and **41*, in relation to **1* and non-functional variant alleles. In this minor investigation only mean group estimates of dose-adjusted serum concentrations of perphenazine are given for the *CYP2D6* genotypes **1/*1*, **1/*9*, **1/*10*, **1/*41*, and **1/null*. For the other included antipsychotics, there were too few data in each subgroup of the reduced function variant alleles to assess similar group estimates.

Age, gender and sampling time were included in the mixed model analyses as potential covariates. Group estimates are presented as mean values with 95% confidence intervals. Statistical significance was considered as $p < 0.05$. SPSS Software version 21.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analyses, and GraphPad Prism version 6 (GraphPad Software Inc., La Jolla, CA USA) was used for graphical presentations.

Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in <http://www.guidetopharmacology.org>, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY.

RESULTS

Initially, the number of serum concentration measurements (number of patients given in parenthesis) of the antipsychotics investigated from *CYP*-genotyped patients available from the TDM database, and fulfilling the inclusion criteria, were 142 (65) for flupentixol, 362 (146) for haloperidol, 611 (204) for perphenazine, and 751 (263) for zuclopenthixol. After exclusion of patients carrying multiplied, functional *CYP2D6* alleles, patients co-medicated with drugs inhibiting or inducing the metabolism of the antipsychotics investigated, and patients with possible noncompliance, the remaining number of serum concentration measurements (number of patients given in parenthesis) included in the study were as follows; flupentixol: 115 (53); haloperidol: 320 (137); perphenazine: 564 (185); and zuclopenthixol: 658 (248) (Table 2). Pharmacokinetically interacting drugs co-medicated with the antipsychotics investigated, which resulted in exclusion of measurements, were the *CYP2D6* inhibitors [bupropion](#), [fluoxetine](#), [paroxetine](#), and [levomepromazine](#) (daily doses of 100 mg or above), the *CYP3A4* inhibitor [verapamil](#), and the *CYP3A4* inducers [carbamazepine](#) and [phenobarbital](#).

The greatest impact of *CYP2D6* was seen for perphenazine, where mean dose-adjusted serum concentrations were 3.9-fold and 1.6-fold higher in *CYP2D6* PMs and IMs, respectively, compared with NMs ($p<0.001$ and $p<0.01$, Table 3, Figure 1C). The magnitude was less for zuclopenthixol, although significant, with mean dose-adjusted serum concentrations about 1.5-fold and 1.3-fold higher in *CYP2D6* PMs and IMs, respectively, compared with NMs ($p<0.01$ and $p<0.001$, Table 3, Figure 1D). There was only a minor (20%) difference in haloperidol mean dose-adjusted serum concentrations between *CYP2D6* IMs and NMs ($p<0.05$), and no significant impact of *CYP2D6* on serum levels of flupentixol (Table 3, Figure 1B and 1A, respectively). Data on each genotype group included in the above mentioned phenotypes are given in Supplementary Figure 1 and Supplementary Table 1.

The mean absolute serum concentration of perphenazine was 2.5-fold higher in *CYP2D6* PMs compared with NMs ($p<0.001$, Table 3). For the other antipsychotics investigated, there were no significant differences in mean absolute serum concentrations between the *CYP2D6* phenotype subgroups.

Regarding mean daily dosage, the only significant difference between *CYP2D6* phenotype subgroups was seen for perphenazine, where PMs had about 38% lower mean daily dosage compared with NMs ($p<0.01$, Table 3).

In the mixed model analyses, the effects of age were 0.0218 nM/mg (95% CI 0.000512-0.0430, $p=0.045$), 0.00132 nM/mg (95% CI -0.00499-0.00764, $p=0.680$), 0.00147 nM/mg (95% CI -

0.0000310-0.00296, $p=0.055$), and 0.0100 nM/mg (95% CI 0.00255-0.0175, $p=0.009$) per year for flupentixol, haloperidol, perphenazine, and zuclopenthixol, respectively.

Data on smoking habits (smoking/non-smoking) were only available for a subset of the included samples; flupentixol (19/16); haloperidol (72/56); perphenazine (74/58); and zuclopenthixol (154/67). Smoking had no impact on dose-adjusted serum concentrations of the investigated antipsychotics (Supplementary Table 2).

For perphenazine serum concentrations, data on *CYP2D6**1/*red* divided in the subgroups *1/*9, *1/*10, and *1/*41 are shown in Figure 2 together with *1/*1 and *1/*null*. In our data material, the genotype *1/*41 is seen in 20 of 185 perphenazine users (130 of 564 samples) and appears to have a similar impact on dose-adjusted serum concentrations of perphenazine as *1/*null*. Although few measurements, *1/*9 and *1/*10 are closer to the *1/*1 genotype compared to *1/*41.

DISCUSSION

The results of this study show that CYP2D6 is important for the metabolism of perphenazine and zuclopenthixol, but is of minor or no importance to haloperidol and flupentixol.

The approximately 3.9-fold and 1.5-fold higher mean dose-adjusted serum concentrations of perphenazine and zuclopenthixol in CYP2D6 PMs compared with NMs in the present study is roughly in consistency with previous findings [9, 10]. The difference in serum level between the two drugs when comparing phenotypes, suggests a higher contribution of CYP2D6 to the metabolism of perphenazine. Nonetheless, dose adjustments according to CYP2D6 genotype should be performed for both of the drugs to reduce the risk of concentration-dependent adverse drug reactions. Studies have shown that CYP2D6 PMs have an increased risk of adverse effects, like over-sedation and extrapyramidal symptoms, when using perphenazine [14, 15]. For zuclopenthixol, there is less evidence linking CYP2D6 PMs to adverse effects, but neurologic adverse effects tend to be more frequent in patients with non-functional CYP2D6 alleles [15]. Further studies should attempt to evaluate the clinical outcome of the CYP2D6 PM phenotype in patients using antipsychotics metabolized by CYP2D6.

The clinical importance of the study results could also be considered by comparing the changes in serum concentrations to the reference limits. For zuclopenthixol, the absolute serum concentrations, also in the PM group, are still well within the reference range, which according to the AGNP is very wide (up to 125 nmol/L [6]). Still, it could be of clinical importance to know that the dose-adjusted serum concentrations are increased by 50% in these patients, as side effects could also occur within the reference range. For perphenazine, however, the absolute serum concentrations in the PM group are more close to the upper reference limit (6 nmol/L according to the AGNP [6]), and the large expected increase in serum concentrations must be considered highly clinically relevant.

For haloperidol and flupentixol, the results suggest that other enzymes than CYP2D6 (e.g. CYP3A4) are mainly involved in the metabolism. There have been some uncertainties regarding CYP2D6 contribution to haloperidol metabolism. Based on studies on steady-state concentration, CYP2D6 genotype mainly influence reduced haloperidol concentration as shown by Patteet et al. 2016, Pan et al. 1999 and Brockmüller et al. 2002 [11, 16, 17]. Unfortunately, our method does not include measurements on reduced haloperidol. However, in these studies, there were no or minor effects of CYP2D6 genotype on the dose-adjusted concentration of haloperidol [11, 16, 17], which is in line with our results.

We also found a significantly higher mean absolute serum concentration in perphenazine CYP2D6 PMs compared with NMs. Although the mean dose-adjusted serum concentration of perphenazine was 3.9-fold higher in PMs compared with NMs, doses were only reduced by 38% in

PMs. This shows that lacking the CYP2D6 enzyme is not compensated for by a sufficient dose reduction of perphenazine to account for the increased systemic exposure in these patients. This could be visualised by the fact that in the PM group of perphenazine users, 30% of the serum samples showed absolute values above the upper level of the reference range (1-6 nmol/L according to the AGNP [6]), while this was the case for 18% of the serum samples in the NM group.

The lack of significance of cigarette smoking on the dose-adjusted serum concentrations of flupentixol, haloperidol, perphenazine and zuclopenthixol are not surprising. The enzyme CYP1A2 is induced by cigarette smoking, but CYP1A2 does not seem to be particularly involved in the metabolism of any of these four antipsychotics [1-6]. However, there are some pharmacokinetic studies showing significantly lower dose-adjusted plasma concentrations of haloperidol and perphenazine in smokers compared with non-smokers [16, 18, 19]. We did not find corresponding results in our TDM material, with more than fifty samples in each group for both drugs. Although only available for a smaller part of the material, the lack of smoking impact indicates that CYP1A2 is not the major enzyme involved in the metabolism of the drugs investigated.

In the present study, we have not corrected for variation in metabolic capacity of other polymorphic enzymes, such as [CYP2C9](#) or [CYP2C19](#). However, CYP2C9 and CYP2C19 are not known to be particularly involved in the metabolism of the four antipsychotics investigated. On the other hand, CYP3A4 are involved in the metabolism of at least some of these antipsychotics [1-6], but for the variation in enzyme capacity of CYP3A4, environmental factors are of much greater importance than genetic polymorphism.

Several of the included patients used more than one of the four antipsychotics. However, this has probably not affected the results. To our knowledge, there are no known pharmacokinetic interactions between flupentixol, haloperidol, perphenazine and zuclopenthixol.

Overall, age seems to be of relatively little importance to the serum levels of the investigated antipsychotics. The modest impact of age on dose-adjusted serum concentrations in the present study is in contrast to previous findings for several other psychotropic drugs [20-23]. According to our results, dose adjustments for age is only required to a relatively small extent using these antipsychotics.

Since perphenazine is the one among these four antipsychotics where reduced and no CYP2D6 enzyme function has most impact on the serum level, and where we had most patients with the *41 allele, we chose this drug to investigate the difference between the reduced function alleles *9, *10 and *41. Our data on the dose-adjusted serum concentrations of perphenazine obtained by patients with the genotypes *CYP2D6**1/*9, *1/*10 or *1/*41 shown in Figure 2, imply that the *41 allele results in a more reduced enzyme function compared with *9 and *10. This is in contrast to the activity scores assigned to these reduced function variant alleles by the Clinical Pharmacogenetics

Implementation Consortium and the Dutch Pharmacogenetics Working Group, where **10* is thought to result in a more reduced enzyme function as compared with **41*, recently published as an expert panel consensus [8]. There are high allele frequencies of *CYP2D6*41* in many European countries, and a study by Haslemo et al. has shown that in *CYP2D6*-genotyped patients on [venlafaxine](#) (n=1003), carriers of *CYP2D6*41* had significantly lower metabolism to [O-desmethylvenlafaxine](#) than carriers of *CYP2D6*9* and **10*, measured as the O/N-desmethylvenlafaxine ratio [24]. From naturalistic data of both perphenazine and venlafaxine, we now have evidence that *CYP2D6*41* results in a more reduced enzyme function and is closer to the non-functional alleles compared with the **10* variant.

The naturalistic study setting, where data was acquired retrospectively, is associated with some methodological limitations. The included serum concentration measurements sampled 10-26 hours after the last dose intake were assumed to be at steady-state, as this is regular practice by use of TDM. Further, in our data material, a small proportion (11%) of the requisition forms was not available for identifying concurrent use of potentially interacting drugs or herbal agents, or known poor patient adherence. Other unknown confounders could also have affected the study results. In addition, patients carrying multiplied, functional *CYP2D6* alleles were not included in the study, and we had no information available on whether the physicians were aware of the patients' *CYP2D6* genotype or not before dosing of the antipsychotics. Furthermore, for flupentixol and haloperidol, there were few samples included and although the differences between phenotype subgroups seem to be small when assessing the estimates, all of these uncertainties together have the potential to affect the results of the study. However, for perphenazine and zuclopenthixol, the relatively high sample number included probably outweighs the disadvantages. Another strength of the study was the application of multivariable mixed model analyses, which enabled inclusion of multiple serum concentration measurements from the same individuals.

In conclusion, this study shows that the genotype of *CYP2D6* is crucial for the serum concentration level of perphenazine and zuclopenthixol, but not for haloperidol and flupentixol. For perphenazine, the mean dose-adjusted serum concentrations were 3.9-fold and 1.6-fold higher in *CYP2D6* genotypes included in the PM and IM phenotypes, respectively, compared with NMs. For zuclopenthixol, PMs and IMs had about 1.5-fold and 1.3-fold higher serum levels than NMs, respectively. Further, the *CYP2D6*41* allele appears to have a reduced function close to non-functional variant alleles, as shown in the perphenazine material. Clinicians should be aware of the patient genotype when prescribing perphenazine and zuclopenthixol, and adjust the dose according to the patients' *CYP2D6* metabolizing capacity.

COMPETING INTERESTS

There are no competing interests to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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TABLE 1. Included alleles in the CYP2D6 phenotype subgroups

CYP2D6 subgroup	Allele combinations
NM	<i>*1/*1, *1/red</i>
IM	<i>*1/null, red/red, null/red</i>
PM	<i>null/null</i>

NM normal metabolizer, IM intermediate metabolizer, PM poor metabolizer; status derived from CYP2D6 genotype
red (i.e. variant allele encoding reduced enzyme function): **9, *10, *41*
null (i.e. variant allele encoding no enzyme function): **3, *4, *5, *6*

TABLE 2. Characteristics of the study population

Antipsychotic drugs	CYP2D6 subgroup	Samples (% male)	Patients	Age, years ^a
Flupentixol	NM	68 (41)	28	49 (19-87)
	IM	39 (28)	21	46 (19-91)
	PM	8 (50)	4	31 (27-77)
Haloperidol	NM	155 (48)	84	65 (18-97)
	IM	153 (27)	47	63 (20-96)
	PM	12 (42)	6	63 (28-68)
Perphenazine	NM	316 (35)	98	45 (17-95)
	IM	179 (40)	67	52 (18-87)
	PM	69 (39)	20	52 (26-79)
Zuclopenthixol	NM	342 (43)	135	49 (19-86)
	IM	268 (47)	98	42 (16-89)
	PM	48 (48)	15	46 (24-60)

^aData is presented as median (range) of all serum sample registrations.

NM normal metabolizer, IM intermediate metabolizer, PM poor metabolizer; status derived from CYP2D6 genotype

TABLE 3. Dose-adjusted serum concentrations, absolute serum concentrations and daily doses of the included antipsychotic drugs in different CYP2D6 phenotype subgroups

Antipsychotic drugs	CYP2D6 subgroup	Dose-adjusted serum concentration, nmol/L/mg/day ^a	Ratio versus CYP2D6 NM	P value versus CYP2D6 NM	Absolute serum concentration, nmol/L ^a	P value versus CYP2D6 NM	Drug dosage, mg/day ^a	P value versus CYP2D6 NM
Flupentixol	NM	2.18 (1.65-2.70)	-	-	8.52 (6.35-10.7)	-	4.08 (2.39-5.76)	-
	IM	1.91 (1.30-2.53)	0.88	0.502	5.65 (3.02-8.27)	0.089	3.84 (1.88-5.81)	0.853
	PM	2.16 (0.793-3.52)	0.99	0.978	5.20 (-0.395-10.8)	0.268	1.87 (-2.49-6.23)	0.340
Haloperidol	NM	1.35 (1.18-1.52)	-	-	5.37 (4.30-6.43)	-	4.32 (3.61-5.03)	-
	IM	1.62 (1.40-1.84)	1.20	<0.05	5.40 (4.02-6.79)	0.968	3.89 (2.97-4.82)	0.467
	PM	1.84 (1.24-2.43)	1.36	0.122	3.16 (-0.628-6.96)	0.270	1.81 (-0.745-4.36)	0.062
Perphenazine	NM	0.130 (0.094-0.166)	-	-	2.15 (1.53-2.78)	-	15.8 (14.1-17.4)	-
	IM	0.205 (0.162-0.249)	1.58	<0.01	2.99 (2.23-3.75)	0.091	13.4 (11.4-15.3)	0.062
	PM	0.505 (0.427-0.584)	3.88	<0.001	5.30 (3.95-6.65)	<0.001	9.87 (6.34-13.4)	<0.01
Zuclopenthixol	NM	1.45 (1.28-1.61)	-	-	20.8 (18.0-23.6)	-	15.2 (13.7-16.8)	-
	IM	1.94 (1.75-2.12)	1.34	<0.001	24.4 (21.1-27.7)	0.107	13.9 (12.1-15.7)	0.267
	PM	2.10 (1.64-2.56)	1.45	<0.01	29.0 (20.8-37.2)	0.064	15.8 (11.3-20.4)	0.799

^aData is presented as mean (95% confidence interval).

NM normal metabolizer, IM intermediate metabolizer, PM poor metabolizer; status derived from CYP2D6 genotype

FIGURE 1.

Dose-adjusted serum concentrations in different CYP2D6 phenotype subgroups of **A** flupentixol, **B** haloperidol, **C** perphenazine, and **D** zuclopenthixol patient samples. The red lines are the mean group estimates given in Table 3. NM normal metabolizer, IM intermediate metabolizer, PM poor metabolizer; status derived from CYP2D6 genotype.

FIGURE 2.

Dose-adjusted serum concentrations of perphenazine in different CYP2D6 genotype subgroups. The number of serum concentration measurements is given for each genotype subgroup (number of patients given in parenthesis). The red lines are the mean group estimates from the mixed model analysis; 0.117 nM/mg/day for *1/*1; 0.083 for *1/*9; 0.053 for *1/*10; 0.165 for *1/*41; and 0.180 for *1/null, and the respective 95% confidence intervals are 0.075-0.158, -0.069-0.235, -0.286-0.392, 0.089-0.240, and 0.132-0.227. *null* (i.e. variant allele encoding no enzyme function): *3, *4, *5, *6.

SUPPLEMENTARY FIGURE 1. Dose-adjusted serum concentrations in different CYP2D6 genotype groups of **A** flupentixol, **B** haloperidol, **C** perphenazine, and **D** zuclopenthixol patient samples. *red* (i.e. variant allele encoding reduced enzyme function): **9*, **10*, **41*; *null* (i.e. variant allele encoding no enzyme function): **3*, **4*, **5*, **6*.

SUPPLEMENTARY TABLE 1. Dose-adjusted serum concentrations, absolute serum concentrations and daily doses of the included antipsychotic drugs in different *CYP2D6* genotype subgroups

Antipsychotic drugs	<i>CYP2D6</i> subgroup	Dose-adjusted serum concentration, nmol/L/mg/day ^a	<i>P</i> value versus <i>CYP2D6</i> *1/*1	Absolute serum concentration, nmol/L ^a	<i>P</i> value versus <i>CYP2D6</i> *1/*1	Drug dosage, mg/day ^a	<i>P</i> value versus <i>CYP2D6</i> *1/*1
Flupentixol	*1/*1	1.98 (1.41-2.55)	-	7.94 (5.42-10.45)	-	3.97 (2.06-5.88)	-
	*1/red	2.96 (1.71-4.20)	NS	8.40 (3.02-13.8)	NS	3.96 (-0.231-8.16)	NS
	*1/null	1.78 (1.12-2.44)	NS	5.06 (2.08-8.05)	NS	4.13 (1.92-6.34)	NS
	red/red	4.09 (1.59-6.59)	NS	6.66 (-3.07-16.4)	NS	1.45 (-7.21-10.1)	NS
	null/red	1.52 (-0.093-3.14)	NS	4.81 (-3.12-12.7)	NS	2.44 (-2.88-7.77)	NS
	null/null	2.10 (0.779-3.43)	NS	4.77 (-1.01-10.5)	NS	1.78 (-2.69-6.24)	NS
Haloperidol	*1/*1	1.31 (1.11-1.50)	-	5.51 (4.28-6.75)	-	4.42 (3.60-5.23)	-
	*1/red	1.55 (1.23-1.86)	NS	4.99 (2.95-7.02)	NS	3.59 (2.25-4.93)	NS
	*1/null	1.67 (1.42-1.92)	NS	5.94 (4.35-7.53)	NS	4.08 (3.02-5.14)	NS
	red/red	2.01 (1.00-3.02)	NS	4.77 (-1.52-11.0)	NS	3.25 (-1.08-7.57)	NS
	null/red	1.46 (0.969-1.95)	NS	3.57 (0.477-6.66)	NS	2.64 (0.547-4.72)	NS
	null/null	1.86 (1.26-2.46)	NS	3.21 (-0.583-7.00)	NS	1.70 (-0.858-4.25)	NS
Perphenazine	*1/*1	0.124 (0.081-0.167)	-	2.14 (1.41-2.87)	-	16.8 (14.9-18.8)	-
	*1/red	0.138 (0.071-0.204)	NS	2.28 (1.15-3.41)	NS	13.7 (10.7-16.7)	NS
	*1/null	0.183 (0.135-0.231)	NS	2.88 (2.06-3.70)	NS	14.4 (12.2-16.5)	NS
	red/red	0.480 (0.285-0.675)	<0.01	8.69 (5.41-12.0)	<0.01	14.5 (5.72-23.3)	NS
	null/red	0.223 (0.112-0.333)	NS	1.82 (-0.063-3.71)	NS	8.54 (3.55-13.5)	<0.05
	null/null	0.504 (0.427-0.580)	<0.001	5.29 (4.01-6.58)	<0.01	10.0 (6.56-13.5)	<0.05
Zuclopenthixol	*1/*1	1.38 (1.20-1.56)	-	20.7 (17.4-24.0)	-	16.1 (14.4-17.9)	-
	*1/red	1.68 (1.36-2.01)	NS	21.0 (15.0-26.9)	NS	12.1 (8.84-15.3)	NS
	*1/null	1.82 (1.61-2.02)	<0.05	23.9 (20.2-27.6)	NS	14.3 (12.3-16.3)	NS
	red/red	2.94 (1.99-3.89)	<0.05	32.8 (15.8-49.9)	NS	11.8 (2.71-20.9)	NS
	null/red	2.34 (1.90-2.79)	<0.01	24.7 (16.5-32.9)	NS	11.9 (7.31-16.4)	NS
	null/null	2.11 (1.65-2.56)	NS	29.0 (20.7-37.2)	NS	15.8 (11.3-20.3)	NS

^aData is presented as mean (95% confidence interval). *P* values are Sidak-adjusted due to multiple comparisons.

red (i.e. variant allele encoding reduced enzyme function): *9, *10, *41

null (i.e. variant allele encoding no enzyme function): *3, *4, *5, *6

NS no significant difference

SUPPLEMENTARY TABLE 2. Dose-adjusted serum concentrations of smoking and non-smoking patients for flupentixol, haloperidol, perphenazine and zuclopenthixol

Drug	Smoking habits	Samples (male/female)	Dose-adjusted serum concentration, nmol/L/mg/day ^a	P value versus non-smoking
Flupentixol	Smoking	19 (14/5)	1.80	NS
	Non-smoking	16 (0/16)	2.46	-
Haloperidol	Smoking	72 (35/37)	1.30	NS
	Non-smoking	56 (22/34)	1.46	-
Perphenazine	Smoking	74 (35/39)	0.14	NS
	Non-smoking	58 (28/30)	0.17	-
Zuclopenthixol	Smoking	154 (78/76)	1.53	NS
	Non-smoking	67 (30/37)	1.58	-

^aData is presented as mean (95% confidence interval).

Samples lacking information on smoking habits are not included in the table.

NS no significant difference