# Phenoconversion of CYP2D6 by inhibitors modifies aripiprazole exposure

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**Abstract**

The efficacy of aripiprazole therapy and the risk of adverse reactions are influenced by substantial inter-individual variability in aripiprazole metabolizing capacity. *In vitro* studies assigned the potential role in aripiprazole metabolism to CYP2D6 and CYP3A enzymes; therefore, the association between the steady-state aripiprazole plasma concentrations and patients' CYP2D6- and CYP3A-status (*CYP2D6*, *CYP3A4*, *CYP3A5* genotypes and CYP3A4 expression) and/or co-medication with CYP-function modifying medications has been investigated in 93 psychiatric patients on stable aripiprazole therapy. The patients' *CYP2D6* genotype had a major effect on aripiprazole plasma concentrations, whereas the contribution of *CYP3A* genotypes and CYP3A4 expression to aripiprazole clearance was considered to be minor or negligible. The role of CYP3A4 expression in aripiprazole metabolism did not predominate even in the patients with non-functional *CYP2D6* alleles. Furthermore, dehydroaripiprazole exposure was also *CYP2D6* genotype dependent. Dehydroaripiprazole concentrations were comparable with aripiprazole levels in patients with functional *CYP2D6* alleles, and 35% or 22% of aripiprazole concentrations in patients with one or two non-functional *CYP2D6* alleles, respectively. The concomitant intake of CYP2D6 inhibitors, risperidone, metoprolol or propranolol was found to increase aripiprazole concentrations in patients with at least one wild-type *CYP2D6*1* allele. Risperidone and 9-hydroxy-risperidone inhibited both dehydrogenation and hydroxylation of aripiprazole, whereas metoprolol and propranolol blocked merely the formation of the active dehydroaripiprazole metabolite, switching towards the inactivation pathways. Patients' *CYP2D6* genotype and co-medication with CYP2D6 inhibitors can be considered to be the major determinants of aripiprazole pharmacokinetics. Taking into account *CYP2D6* genotype and co-medication with CYP2D6 inhibitors may improve outcomes of aripiprazole therapy.

Keywords: aripiprazole therapy, CYP2D6 phenoconversion, risperidone, metoprolol, propranolol

## Introduction

A patient's response to a drug is highly influenced by his/her drug-metabolizing capacity. Genetic variations of drug-metabolizing enzymes can significantly modify the pharmacokinetics of a drug; thus, genetic polymorphism is considered to account for one of the main sources of inter-individual differences in drug metabolism and eventually in drug response (efficacy and/or safety) [1-3]. The non-functional or increased function mutations in drug-metabolizing enzymes lead to poor or rapid metabolizer phenotypes, whereas the wild-type allele is predicted to be translated to an enzyme with normal function. However, non-genetic factors, such as age, sex, disease and medication, can alter the expression or the activity of drug-metabolizing enzymes; therefore, homozygous wild-type genotype can be transiently switched into poor (or rapid) metabolism due to phenoconversion [4-7]. Consequently, the heritable traits determine only the potential for the expression of functional or non-functional enzyme, and non-genetic factors can give rise to altered phenotypes, resulting in genotype-phenotype mismatch.

The atypical antipsychotic aripiprazole is approved by the European Medicines Agency for treatment of schizophrenia as well as for treatment of moderate to severe manic episodes of bipolar I disorder [8-11]. Aripiprazole has unique receptor-binding properties acting as a partial agonist at dopaminergic $D_2$ and serotonergic 5-$HT_{1A}$ receptors, and as an antagonist at 5-$HT_{2A}$ receptors [8, 9, 12]. It has advantages, such as lower risk of extrapyramidal symptoms, hyperprolactinemia, bodyweight gain, diabetes mellitus and sedation; however, the treatment discontinuation rate associated with aripiprazole inefficacy seems to be higher than that of olanzapine or haloperidol [10, 13-15]. Almost complete dopamine receptor occupancy (>85%) has been observed at the plasma concentration of 100-150 ng/ml, and the optimal serum concentrations that result in the best improvement and no or mild side effects in patients, have been suggested to range between 150 and 300 ng/ml, whereas the risk of moderate to severe adverse effects increases at higher concentrations [16-19]. At the same dose, considerable variability in aripiprazole concentration can occur which can be attributed to the inter-individual differences in aripiprazole metabolism; therefore, therapeutic drug monitoring is recommended [19, 20].

Aripiprazole is extensively metabolized in the liver, forming the pharmacologically active dehydroaripiprazole as well as the inactive *N*-dealkyl-aripiprazole and two monohydroxy-metabolites [10, 21-24]. The major metabolite dehydroaripiprazole displays similar pharmacological properties to the parent compound, contributing to the antipsychotic effect of aripiprazole; therefore, the sum of the parent drug and its dehydro-metabolite is often monitored [10, 17, 22, 25, 26]. CYP2D6 and CYP3A4 are primarily responsible for aripiprazole metabolism, which can entail the potential for high inter-individual variability in aripiprazole pharmacokinetics [17, 25, 27]. CYP2D6 is one of the most polymorphic cytochrome P450 enzymes with more

than 100 alleles identified (https://www.pharmvar.org/htdocs/archive/cyp2d6.htm). In Caucasian populations, several non-functional and reduced function *CYP2D6* alleles as well as the whole *CYP2D6* gene deletion (*CYP2D6*3*, *CYP2D6*4*, *CYP2D6*5*, *CYP2D6*6*, *CYP2D6*10*, *CYP2D6*41*) commonly occur, leading to absent or non-functional protein or decreased expression. *CYP2D6* gene duplication/multiplication of functional alleles, resulting in increased expression and CYP2D6 activity, has also been identified [2]. Genetic polymorphism of *CYP2D6* is one of the main sources of inter-individual differences in metabolism of some CYP2D6 substrates, such as tricyclic antidepressants (desipramine, imipramine, nor-triptyline), fluoxetine, paroxetine, risperidone, venlafaxin, metoprolol [28, 29]; however, non-genetic factors like co-administration of a drug that acts as a CYP2D6 inhibitor can also influence CYP2D6 activity. Concomitant use of selective serotonin reuptake inhibitors (duloxetine, fluoxetine, paroxetine, sertraline) has been demonstrated to decrease aripiprazole metabolizing activity [30]. Therefore, both genetic variability and co-medication with CYP2D6 inhibitors are recommended to take into account in aripiprazole dosing [18, 30, 31]. CYP3A4 activity also displays high inter-individual variability, which is partly attributed to genetic factors. *CYP3A4*1B* allele is assumed to increase CYP3A4 transcription; however, the clinical significance of *CYP3A4*1B* to CYP3A4 activity is doubtful [32, 33]. *CYP3A4*22* has been demonstrated to display low hepatic CYP3A4 expression, resulting in decreased CYP3A4 activity [34]. It has been suggested that the association between *CYP3A4*22* and pharmacokinetic behaviour of CYP3A-substrates could be evaluated in combination with *CYP3A5* genotype [35]. *CYP3A5*3* allele results in splicing defect and non-functional CYP3A5 enzyme. Those individuals who have functional CYP3A5 enzyme are presumed to metabolize some CYP3A-substrates more rapidly than CYP3A5 non-expressers. Although genetic polymorphisms of *CYP3A* can influence CYP3A activity, non-genetic factors, especially co-medication resulting in transient poor or rapid metabolism, may have much higher impact on aripiprazole pharmacokinetics. The potent CYP3A4 inducer carbamazepine has been reported to substantially decrease the plasma concentrations of both aripiprazole and its dehydro-metabolite, whereas CYP3A4 inhibitors (itraconazole, fluvoxamine) seem to increase aripiprazole exposure [23, 31, 36].

For the estimation of patients' aripiprazole-metabolizing capacity, *CYP2D6* genotype, CYP3A-status (*CYP3A4/5* genotype and CYP3A4 expression) and co-medication with CYP2D6 and CYP3A4 inhibitors or with CYP3A4 inducers should be considered. Although CYP genotypes can be simply identified by *CYP2D6*, *CYP3A4* and *CYP3A5* genotyping, the crucial task is the assessment of hepatic CYP3A4 activity. We have previously described a complex diagnostic system (CYPtest$^{TM}$) that estimates CYP3A-metabolizing capacity by CYP3A4 expression in leukocytes. CYP3A4 mRNA levels in leukocytes were demonstrated to inform about the hepatic CYP3A4 activity [37]. The goals of the present study were to estimate the association between the patients' drug-metabolizing capacity (CYP expression and CYP genotypes) and their aripiprazole therapy (dose,

aripiprazole plasma levels), and to analyze the influence of genetic factors and the non-genetic factor co-medications on aripiprazole exposure. Taking into account patients' CYP-status and current co-medication can contribute to the improvement of patients' personalized medication and can predict the risk of outlying from the therapeutic concentration range.

## Materials and methods

### Patients

Ninety-three inpatients at the Department of Psychiatry and Psychotherapy, Semmelweis University (Budapest, Hungary) were enrolled, and written informed consent was obtained from all participants. The study was approved by the Hungarian Committee of Science and Ethics. Adult patients (≥18 years) on stable aripiprazole dose for more than four weeks, displaying two identical concentrations at an interval of 14 days were included in the study. Patients' demographic data and medication was recorded (Table 1). The aripiprazole therapy was applied according to the conventional clinical protocol, initiated at low dosage (7.5 mg/day), and subsequently titrated up to the target dose of 10-30 mg/day until the optimal response was achieved. Patients' improvement was measured by repeated psychiatric interviews and reduction of both the total scores of PANSS (positive and negative syndrome scale) and CGI (clinical global impression). Aripiprazole dosage was recorded for four weeks before blood sampling for testing patients' CYP-status and for drug assay.

### Assaying CYP-status

Patients' CYP-status was determined by *CYP2D6*, *CYP3A4* and *CYP3A5* genotyping and by analyzing CYP3A4 expression in leukocytes. Genomic DNA and leukocytes were isolated from the peripheral blood samples as previously described by Temesvári et al. [37]. Hydrolysis single-nucleotide polymorphism analysis for *CYP2D6*3*, *CYP2D6*4*, *CYP2D6*6*, *CYP2D6*10*, *CYP2D6*41*, *CYP3A4*1B*, *CYP3A4*22* and *CYP3A5*3* was performed using TaqMan probes (BioSearch Technologies, Novato, CA). *CYP2D6*5* (gene deletion) and *CYP2D6* duplication/multiplication were analyzed by TaqMan Copy Number Assay (ThermoFisher Scientific, Waltham, MA). Allele specific identification of *CYP2D6* duplication was determined as previously described [38].

For assaying CYP3A4 expression, RNA was isolated from leukocytes, reverse transcribed into single-stranded cDNA using the Maxima First Strand cDNA Synthesis Kit (ThermoFisher Scientific), and real-time PCR was performed using KAPA Fast Probes Mastermix (KAPA Biosystems, Cape Town, South Africa) and TaqMan probes (Microsynth AG, Balgach, Switzerland). The quantities of CYP3A4 mRNA relative to that of the housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were determined. GAPDH expression is constant in all cells and independent of experimental conditions; therefore, its expression was set to

1 and CYP3A4 mRNA levels were normalized by GAPDH expression. Three categories of CYP3A4 expression were applied to describe low, normal and high expressers. The cut-off values for CYP3A4 mRNA levels in leukocytes have been previously established on the basis of the cut-off values for the hepatic CYP3A4 enzyme activities (nifedipine oxidation or midazolam 1'- and 4-hydroxylation). The cut-off values for CYP3A4 ($10^{-6}$ and $10^{-4}$) allowed a distinction between low, normal and high expressers [37].

**Plasma concentrations of aripiprazole**

The blood samples of patients on stable aripiprazole therapy were taken between 8 and 9 am before the morning dose of aripiprazole (generally 12 hr since last drug intake). The steady-state condition was confirmed by reviewing the patient's therapeutic history. The blood samples were taken at the same time for CYPtesting and for therapeutic drug monitoring. The analytical assay was validated for routine drug monitoring. The steady-state plasma concentrations of aripiprazole and dehydroaripiprazole were determined by LC-MS/MS (liquid chromatography-tandem mass spectrometry). Chromatographic separation was performed using an Inertsil ODS-4 (75×2.1 mm, 3 µm) column (GL Sciences Inc., Tokyo, Japan) and mobile phases of acetonitrile and 0.1% formic acid in gradient running mode. The samples in triplicates were analyzed using positive electrospray ionization and multiple reaction monitoring (MRM) mode for quantitation of the parent compound and its active metabolite (*m/z* [mass/charge] 448/285 and 448/176 for aripiprazole; *m/z* 446/285 and 446/216 for dehydroaripiprazole). Calibration plots for aripiprazole and dehydroaripiprazole were linear over the range of 1-1000 and 1-500 ng/ml, respectively. The intraday and interday precision was less than 8%. Normalized aripiprazole concentrations were calculated by dividing the concentration values by the corresponding 24-h dose on a mg/kg basis.

**Inhibition of aripiprazole metabolism**

*In vitro* metabolite formation from aripiprazole was determined in human liver microsomes. Hepatic microsomal fraction was isolated from three tissue donors by differential centrifugation [39]. Protein content of microsomes was determined by the method of Lowry et al. [40], with bovine serum albumin as the standard. For aripiprazole metabolism, the incubation mixture contained the NADPH-generating system (1 mM NADPH, 10 mM glucose 6-phosphate, 5 mM $MgCl_2$ and 2 units/ml glucose 6-phosphate-dehydrogenase), human liver microsomes (1 mg protein/ml) and aripiprazole. After 60-min incubation, reactions were terminated by the addition of ice-cold acetonitrile. The amounts of aripiprazole metabolites (dehydroaripiprazole, *N*-dealkyl-aripiprazole, monohydroxy-aripiprazole) produced were determined by LC-MS/MS. The analytical conditions were the same as described for the parent compound and dehydroaripiprazole measured in human plasma except for the MRM transitions (*m/z* 231/188 and 231/152 for *N*-dealkyl-aripiprazole; *m/z* 464/285 and 464/234 for

monohydroxy-aripiprazole). Inhibition of metabolite formation from aripiprazole was carried out in the absence and presence of risperidone, 9-hydroxy-risperidone, metoprolol or propranolol. $K_i$ values (inhibition constants) were determined by using different concentrations of aripiprazole (10, 25, 50 μM) and potential CYP2D6 inhibitors (1-50 μM). $K_i$ values were calculated from Dixon plots of 1/velocity versus inhibitor concentration at the three aripiprazole concentrations. The type of inhibition and the apparent $K_i$ values were estimated from the intercept of three lines of Dixon plots and expressed as the mean±SD of the intercepts.

**Data analysis**

Statistical significance of CYP3A4 expression, *CYP2D6* and *CYP3A5* genotypes as covariates of aripiprazole plasma concentrations was analysed by principal component analysis and partial least squares (PLS) modelling (SIMCA, MKS Umetrics AB, Umea, Sweden). Principal components are defined as linear combination of original variables and ordered by their contribution degree to the overall data set variability. PLS model is a multiple regression model that first transforms independent (input) and output variables with principal component analysis to remove correlations between the variables and then calculates optimal linear model in iterative procedure. Model coefficients analysis reveals original variables that provide significant information for the output variable prediction. Predicted *vs* measured plot shows how well the model describes the data. Departure from the diagonal line indicates biased model and dispersion of points around the diagonal line shows how much of the total data variability can be explained by the model.

The data of normalized aripiprazole concentrations and the dehydroaripiprazole/parent drug ratios in the patients were expressed as the median and range or mean±SD. Between-group-differences were calculated by the use of Kruskal–Wallis analysis of variance followed by Dunn's multiple comparisons test. For the evaluation of co-medication with CYP2D6 inhibitors, the prevalence of outliers with exaggerated concentrations of aripiprazole (> 300 ng/ml) was compared in patients carrying *CYP2D6*1* by the use of Fisher's exact test. A *P* value of <0.05 was considered to be statistically significant.

**Results**

**CYP-status and aripiprazole exposure**

Of 93 patients, 52 carried at least one non-functional (*nf*) or reduced function (*red*) *CYP2D6* allele (*nf*: *CYP2D6*3*, *CYP2D6*4*, *CYP2D6*4×N*, *CYP2D6*5*, *CYP2D6*6; red: CYP2D6*10*, *CYP2D6*41*), and 5 patients had functional *CYP2D6* duplication (*CYP2D6*1×N*), presumably resulting in increased CYP2D6 expression. Strong association was found between the patients' *CYP2D6* genotype and the normalized aripiprazole plasma concentrations (Fig. 1A). Significantly higher aripiprazole levels were observed in the patients with non-functional and reduced function *CYP2D6* alleles (*CYP2D6*1/nf, CYP2D6*1/red* or

*CYP2D6nf/nf*, *CYP2D6nf/red*) than in those carrying homozygous wild-type genotype (*CYP2D6*1/*1*) or *CYP2D6* duplication (*CYP2D6*1/*1×N*) (1421±384.3, 1379±466.5 or 2221±1003.7, 2191±682.7 *vs* 494.3±158.3 or 543.6±148.7 (ng/ml)/(mg dose/kg bw), $P<0.0001$). Furthermore, significant differences in aripiprazole levels were found between the carriers of one and two non-functional or reduced function *CYP2D6* alleles ($P<0.01$). While the normalized aripiprazole concentrations were comparable in the patients with *CYP2D6*1/*1* genotype and in those with *CYP2D6* duplication (*CYP2D6*1/*1×N*); however, further confirmation is needed because of the relatively small number of patients with *CYP2D6*1* duplication. The patients with *CYP2D6*1/nf* genotype displayed similar aripiprazole concentrations to those with *CYP2D6*1/red*; therefore, these patients were grouped as *CYP2D6*1/mut*. Furthermore, aripiprazole metabolizing capacity of the patients carrying *CYP2D6nf/red* was as low as those with *CYP2D6nf/nf*, assigning them to *CYP2D6mut/mut* group. Thereafter, *CYP2D6mut* was applied for *CYP2D6*3*, *CYP2D6*4*, *CYP2D6*4×N*, *CYP2D6*5*, *CYP2D6*6*, *CYP2D6*10* and *CYP2D6*41* alleles.

To clarify the role of CYP3A enzymes in aripiprazole metabolism, the relationship between patients' CYP3A-status and aripiprazole plasma concentrations was also investigated. Patients with *CYP3A4*22* or with *CYP3A4*1B* allele (13/93) were predicted to display permanent low or high CYP3A4 mRNA expression; however, these alleles cannot explain the high inter-individual variability in CYP3A4 activity, and non-genetic factors, resulting in transiently altered metabolic capacity, are considered to substantially modify the expression of functional *CYP3A4* gene. CYP3A4 expression assays revealed that most of the patients (>80%) expressed CYP3A4 at normal level (Table 1). Nevertheless, no association was found between the patients' CYP3A4 expression and the normalized aripiprazole plasma concentrations (Fig. 1B). Furthermore, the steady-state aripiprazole concentrations in the patients with functional CYP3A5 (*CYP3A5*1*) were similar to those in CYP3A5 non-expressers (*CYP3A5*3/*3*). It may be assumed that the role of CYP3A4 in aripiprazole metabolism predominates in patients with one or two non-functional *CYP2D6* alleles. However, no differences in aripiprazole concentrations were observed between low and normal CYP3A4 expresser patients carrying *CYP2D6*1/mut* (1337.5±355.5 *vs* 1430.3±423.2 (ng/ml)/(mg dose/kg bw), $P=0.5729$). While those with *CYP2D6mut/mut* all expressed CYP3A4 at normal level; therefore, no conclusion could be drawn regarding the contribution of CYP3A4 to aripiprazole clearance.

The plasma concentrations of dehydroaripiprazole widely varied in the patients (4.94 – 284 ng/ml); however, its formation appeared to be associated merely with the patients' *CYP2D6* genotype (Fig. 2), whereas the dehydroaripiprazole/aripiprazole concentration ratios were comparable in the patients expressing CYP3A4 at low, normal or high level (data not shown). The patients with homozygous wild-type genotype (*CYP2D6*1/*1*) or with *CYP2D6* duplication (*CYP2D6*1/*1×N*) displayed significantly higher dehydroaripiprazole/aripiprazole

ratios than those who carried one or two *CYP2D6mut* alleles (0.9±0,191 or 0.967±0.310 *vs* 0.346±0.099 and 0.232±0.085; $P<0.0001$). Furthermore, small but significant differences in aripiprazole dehydrogenation were observed between the patients with one and with two non-functional/reduced function alleles ($P<0.001$).

To identify the major factors influencing aripiprazole steady-state concentration, the PLS model was constructed with input variables, such as patients' bodyweight, aripiprazole daily dose, patients' CYP3A-status (*CYP3A4* genotype, CYP3A4 expression, *CYP3A5* genotype), *CYP2D6* genotype (Fig. 3A), and with the aripiprazole plasma concentration (ng/ml) as the output variable. The number of input variables was reduced to those with significant contribution regarding the distribution of centred and scaled model coefficients. The patients' *CYP3A4* genotype as an independent input variable was eliminated, because CYP3A4 expression reflects the influence of *CYP3A4* genotype. While *CYP2D6*1/*1* and *CYP2D6*1/*1×N* genotypes were combined, because these genotypes seemed to have similar effect on aripiprazole metabolizing capacity. The final PLS model inputs were aripiprazole dose, patient's bodyweight and *CYP2D6* genotype, creating the following equation:

$$cc_{aripiprazole} = 133.57-0.556*bw+8.77*D+constant_{CYP2D6}$$

where $cc_{aripiprazole}$ is aripiprazole plasma concentration predicted from the model (ng/ml), bw is the patient's bodyweight (kg), D is the daily dose of aripiprazole (mg), and the $constant_{CYP2D6}$ is -132.89 for *CYP2D6*1/*1* or *CYP2D6*1/*1×N* genotypes, 32.52 for *CYP2D6*1/mut*, and 210.94 for *CYP2D6mut/mut*. $R^2$ (0.82) and $Q^2$ (0.8) of the PLS model indicated that the model had very good prediction power. The model also passed permutation test, further indicating that the output was highly sensitive on the input values, and thus had strong predictive power. The values of $R^2$ and $Q^2$ suggested that the majority (>80%) of the aripiprazole predose concentration variability originated from aripiprazole dose, patient's bodyweight and CYP2D6 genotype, whereas less than 20% of the variability remained unexplained by the model (Figure 3B). When removing CYP2D6 genotype from the model, only about 13% of aripiprazole concentration variability could be explained.

**Co-medication and aripiprazole exposure**

Of 93 patients, 15 patients were prescribed aripiprazole as monotherapy, whereas the majority of the patients received concomitant antipsychotic medication (clozapine, haloperidol, quetiapine or risperidone) or anticonvulsant drugs (clonazepam, valproic acid or lamotrigine) as adjunctive therapy. Thirty-six patients received beta-adrenergic blockers (propranolol or metoprolol) and ten were on metformin therapy. Co-medication with drugs known to inhibit CYP2D6 on aripiprazole plasma concentrations was assumed to modify aripiprazole clearance. In the patients with at least one *CYP2D6*1* allele, the concomitant intake of the antipsychotic risperidone and/or of the beta-adrenerg blocker metoprolol or propranolol significantly influenced

the elimination rate of aripiprazole, whereas upon CYP2D6 inhibitor therapy, the normalized aripiprazole levels were unchanged in the patients with two *CYP2D6mut* alleles (Fig. 4). The mean aripiprazole concentrations were approximately 50% higher in patients with *CYP2D6*1/*1* or *CYP2D6*1/*1×N* genotypes ($P<0.0001$) and 20% higher in the patients carrying *CYP2D6*1/mut* ($P<0.05$) as a consequence of CYP2D6 inhibitor co-medication. Furthermore, co-medication of patients carrying wild-type *CYP2D6*1* allele with the CYP2D6 inhibitor risperidone, metoprolol or propranolol increased the prevalence of outlier patients with exaggerated concentrations of aripiprazole. Aripiprazole concentration higher than 300 ng/ml was observed more frequently in the patients under CYP2D6 inhibitor therapy than in those who received no CYP2D6 inhibitor co-medication (8/37 vs 2/44, $P=0.0378$). Independently from CYP2D6 inhibitor co-administration, all patients with two non-functional/reduced function alleles (*CYP2D6mut/mut*) displayed exaggerated aripiprazole concentrations.

Inhibitory effects of risperidone and its main metabolite 9-hydroxy-risperidone as well as of propranolol and metoprolol on aripiprazole metabolism were assayed *in vitro* in human liver microsomes (Table 2). Risperidone and 9-hydroxy-risperidone significantly reduced the formation of dehydroaripiprazole and monohydroxy-aripiprazole, and displayed competitive inhibition with the inhibitory kinetic constants ($K_i$) ranged between 8.4 and 13.9 μM. *N*-Dealkylation of aripiprazole was also inhibited by risperidone; however, the inhibitory constant was about one magnitude higher than for dehydrogenation or hydroxylation pathways. In contrast to risperidone, the beta-blocker metoprolol and propranolol behaved as competitive inhibitors merely towards dehydrogenation of aripiprazole, and did not inhibit hydroxylation and *N*-dealkylation pathways. Substantial differences in the inhibition of dehydroaripiprazole formation were observed between metoprolol and propranolol; the inhibitory constant for propranolol was 3.5-fold higher than for metoprolol, suggesting that metoprolol is a more potent inhibitor of dehydrogenation than propranolol.

Co-medication with valproic acid was also considered as a non-genetic factor that can potentially modify aripiprazole metabolism by up-regulation of CYP3A4 expression. However, in the patients on adjunctive valproic acid therapy (N=11), neither aripiprazole plasma concentration nor CYP3A4 expression differed significantly from those on non-valproic acid therapy (data not shown).

## Discussion

Due to high inter-individual variability of aripiprazole metabolizing enzymes, therapeutic drug monitoring for aripiprazole is recommended to reduce poor response or development of adverse effects [18-20]. CYP2D6 and CYP3A4 have been reported to be involved in aripiprazole metabolism, and the association between *CYP2D6* genotype and steady-state concentrations of aripiprazole has been demonstrated by several authors, whereas the impact of *CYP3A* polymorphisms (e.g. *CYP3A5* and *CYP3A4* genotypes) on aripiprazole

pharmacokinetics has not been clearly proved [31, 41, 42]. Although *CYP3A5*3*, the most frequent allele in Caucasians, is associated with loss of CYP3A5 expression and a consequence of no enzyme activity, and *CYP3A4*1B* and *CYP3A4*22* lead to altered expression of CYP3A4, the substantial inter-individual variability in CYP3A activity cannot be explained exclusively by genetic polymorphisms. CYP3A5 is expressed in the liver at much lower concentration than CYP3A4 (approximately 1% and 30% of the total hepatic CYP content, respectively) [2]; however, the functional CYP3A5 enzyme can display much higher affinity towards certain CYP3A substrates, such as tacrolimus, than CYP3A4, and CYP3A5 expresser patients carrying *CYP3A5*1* can metabolize tacrolimus more rapidly than those with *CYP3A5*3/*3* genotype [43]. Current studies have reported that *CYP3A5* and *CYP3A4* polymorphisms hardly had an impact on aripiprazole pharmacokinetics [44, 45]; however, drawing conclusion from *CYP3A* genotypes on the potential role of CYP3A enzymes in aripiprazole metabolism is considered to be the limitations of these studies. CYP3A4 activity seems to be influenced by non-genetic factors rather than by genetic polymorphisms. The non-genetic factors, such as age, hormonal status or transcriptional induction by CYP3A-inducers, such as carbamazepine or valproic acid, can mask the effect of genetic factors on CYP3A4 expression [2, 46]; therefore, for the categorization of the patients regarding CYP3A4 expression, more useful information can be obtained from CYP3A4 mRNA levels than from *CYP3A4*-genotyping. CYP3A-metabolizing capacity determined by CYP3A4 expression in leukocytes was demonstrated to inform about the hepatic activity towards CYP3A substrates, such as ciclosporin, tacrolimus or clonazepam [43, 47].

Our findings confirmed the results previously reported in the literature that *CYP2D6* genotype has a major impact on aripiprazole plasma concentrations normalized by the dose [42, 44, 45, 48]; however, some overlap in the aripiprazole metabolizing activity between *CYP2D6* genotypes was observed. The aripiprazole metabolizing capacity of *CYP2D6nf/red* patients was as reduced as that of the individuals carrying two non-functional *CYP2D6* alleles (*CYP2D6nf/nf*), despite the fact that intermediate and poor metabolizer phenotypes were assigned to *CYP2D6nf/red* and *CYP2D6nf/nf*, respectively [49]. The aripiprazole plasma concentrations in *CYP2D6*1/nf* genotype group were close to those subjects carrying *CYP2D6*1/red*, but significantly different from those with two functional alleles (*CYP2D6*1/*1*), although these individuals with *CYP2D6*1/nf*, *CYP2D6*1/red* or *CYP2D6*1/**1 were listed in the same normal CYP2D6 metabolizer phenotype. Furthermore, the patients with *CYP2D6*1/*1 ×N* genotype predicted to be ultra-rapid metabolizers displayed aripiprazole concentrations similar to the normal metabolizer patients with *CYP2D6*1/*1*. Our results confirm the findings of Hendset et al. [48] predicting similarly reduced aripiprazole metabolizing activity of *CYP2D6red* and *CYP2D6nf* alleles. The present study was the first that attempted to investigate the association between aripiprazole plasma concentrations and the patients' CYP3A-status (CYP3A4 expression together with *CYP3A5* genotype). CYP3A

activity estimated from the patients' CYP3A-status did not appear to significantly influence aripiprazole clearance. Principal component analysis of the patients' drug-metabolizing capacity parameters as well as of demographic and aripiprazole dosing data also identified *CYP2D6* genotype as the main factor in aripiprazole elimination, whereas the contribution of CYP3A activity to aripiprazole pharmacokinetics may be minor or negligible. Following repeated administration of aripiprazole, dehydroaripiprazole exposure was also found to be *CYP2D6* genotype dependent. Dehydroaripiprazole plasma concentrations were comparable with aripiprazole levels in the patients carrying *CYP2D6*1/*1* or *CYP2D6*1/*1×N* genotype, whereas the patients with one or two non-functional *CYP2D6* alleles displayed dehydroaripiprazole concentrations at 35% or 22% of aripiprazole levels, respectively. This means that in patients with non-functional *CYP2D6* alleles, the higher plasma concentrations of aripiprazole were associated with a decrease in dehydroaripiprazole concentrations.

The potential role of CYP2D6 in aripiprazole clearance, clearly demonstrated by the strong association between the patients' *CYP2D6* genotype and the plasma aripiprazole concentrations or the dehydro-metabolite/aripiprazole ratios, also means that aripiprazole pharmacokinetics can be sensitive to potential CYP2D6 inhibitors. Multi-drug therapy is often applied for patients with psychiatric disorders; thus, combination of antipsychotics and/or of antipsychotics with antidepressants or with drugs for the treatment of comorbid medical conditions can increase the risk of pharmacokinetic drug interactions. Selective serotonin reuptake inhibitors are often prescribed with antipsychotics to treat depressive periods; however, many of them (such as paroxetine, fluoxetine, sertraline or duloxetine) are strong CYP2D6 inhibitors resulting in transient poor metabolism of CYP2D6 substrates due to phenoconversion [30]. Aripiprazole clearance was significantly decreased by co-administration of the CYP2D6 inhibitor paroxetine or fluoxetine [31, 50]. In the present study, none of these selective serotonin reuptake inhibitors was applied because of an increased risk of akathisia. Co-medication with risperidone, metoprolol and propranolol was nevertheless assumed to modify aripiprazole metabolism, because these drugs are CYP2D6 substrates and have the capability to inhibit CYP2D6 activity [51-54]. We have observed that concomitant use of risperidone, metoprolol or propranolol was associated with an increase in aripiprazole concentration. Risperidone was demonstrated to be an inhibitor of aripiprazole dehydrogenation and hydroxylation pathways, displaying inhibitory potencies ($K_i$) similar to that was reported for the CYP2D6-selective bufuralol 1'-hydroxylation (6.9±4.1 μM) [51]. Eap et al. [55] found risperidone to be a weak *in vivo* inhibitor of dextromethorphan *O*-demethylation catalysed by CYP2D6; however, the patients involved in the study received risperidone dose lower than usual. The inhibitory potency of the main risperidone metabolite, 9-hydroxy-risperidone, towards dehydrogenation and hydroxylation of aripiprazole was found to be similar to that of risperidone. This means that the hydroxylation of risperidone did not abolish the inhibition of aripiprazole metabolism. Furthermore, 9-hydroxy-risperidone is a pharmacologically active metabolite and has

been marketed as paliperidone [56]; therefore, co-medication with paliperidone may be expected to increase aripiprazole plasma concentrations as well. The presence of metoprolol or propranolol also decreased aripiprazole metabolism; however, metoprolol was found to be more potent inhibitor than propranolol. Using various CYP2D6-selective substrates, such as bufuralol or dextromethorphan, *in vitro* inhibitory potencies ($IC_{50}$) of metoprolol and propranolol towards CYP2D6 have been reported to be within the same order of magnitude (varied between 2.5 and 9.8 μM) [52-54]. Although metoprolol was concluded to be a clinically not relevant inhibitor of venlafaxin metabolism [57], Kirschbaum et al. [18] have found a significant increase in aripiprazole blood concentration in patients treated with metoprolol which is in line with our results. From *in vitro* inhibition parameters, Turpeinen et al. [54] estimated relatively high *in vivo* CYP2D6 inhibition percentage for propranolol (32%). In the current study, these beta-adrenerg receptor blockers inhibited merely the formation of the active dehydroaripiprazole metabolite, and did not influence hydroxylation and *N*-dealkylation pathways. Since *N*-dealkyl-aripiprazole and hydroxy-aripiprazole are inactive metabolites, the inactivation pathways may become the principal route of aripiprazole metabolism upon co-medication with metoprolol or propranolol, resulting in potential modification of aripiprazole efficacy. Aripirazole has been reported to have a significantly higher risk of akathisia compared to other second generation antipsychotics [58], and beta-adrenerg receptor blockers, such as propranolol or metoprolol, are frequently used in the clinical management of antipsychotics induced akathisia [59]. The fact that co-administration of aripiprazole and beta-blockers is common, underlines the clinical significance of our findings. Co-medication with risperidone, metoprolol or propranolol was found to entail higher prevalence of exaggerated aripiprazole concentrations which may increase the risk of moderate and severe side effects developing more frequently above 300 ng/ml [18].

One of the limitations of the present study is that it investigated the pharmacokinetic interactions of relatively weak CYP2D6 inhibitors with aripiprazole; however, not only the simple co-administration of aripiprazole with risperidone or with beta-blockers, but the multiple combinations of these drugs are also frequent which might substantially reduce CYP2D6 function. Due to the fact that this was a naturalistic study, the number of patients treated with CYP2D6 inhibitors was low; however, the relatively narrow range of aripiprazole blood concentrations (primarily in *CYP2D6*1/*1* group) indicated that these three drugs have similar inhibitory potential on CYP2D6 and on aripiprazole metabolism *in vivo*. For acceptable statistical analysis, we pooled the inhibition data which is another limitation of the present study and further investigation is required to confirm our findings. Although the genetic variations of transporter components responsible for the absorption, distribution and elimination of many drugs (e.g. ABCB1) were not investigated, the present study attempted to clarify the functional role of CYP2D6 and CYP3As as well as to identify some potentially interacting drugs in aripiprazole clearance. Our findings demonstrated that patients' *CYP2D6* genotype primarily

influenced steady-state aripiprazole plasma concentration and dehydroaripiprazole formation, whereas contribution of CYP3A4 to *in vivo* aripiprazole clearance was considered to be minor or negligible. Additionally, co-administration of CYP2D6 inhibitors, such as risperidone, metoprolol or propranolol, was found to modify aripiprazole exposure in patients carrying wild-type *CYP2D6*1* allele. Our results suggest that patients' *CYP2D6* genotype and co-medication with CYP2D6 inhibitors can be considered to be the major determinants of aripiprazole pharmacokinetics. However, further studies should be performed to clearly demonstrate whether taking into account patients' *CYP2D6* genotype and co-medication with CYP2D6 inhibitors can improve outcomes of aripiprazole therapy.

**Conflict of Interest**

The authors declare that they have no conflicts of interest.

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Table 1. Patients' demographic and clinical characteristics

| | | N | % |
|---|---|---|---|
| Patients | | 93 | |
| Sex, male/female | | 41/52 | 44.1/55.9 |
| Diagnosis | | | |
| | Schizophrenia | 85 | 91.4 |
| | Bipolar disorder | 8 | 8.6 |
| Age (year)* | | 31 (18; 65) | |
| Bodyweight (kg)* | | 80 (47; 145) | |
| Aripiprazole daily dose (mg)* | | 15 (5; 30) | |
| Serum levels (ng/ml) | | | |
| | Aripiprazole* | 193 (6.2; 819) | |
| | Dehydroaripiprazole* | 86.8 (4.9; 284) | |
| CYP genotype | | | |
| *CYP2D6* | *CYP2D6*1/*1* | 36 | 38.7 |
| | *CYP2D6*1/nf*** | 26 | 28.0 |
| | *CYP2D6*1/red**** | 14 | 15.0 |
| | *CYP2D6nf/nf*** | 7 | 7.5 |
| | *CYP2D6nf/red**** | 5 | 5.4 |
| | *CYP2D6*1/*1xN* | 5 | 5.4 |
| *CYP3A4* | *CYP3A4*1/*1* | 80 | 86 |
| | *CYP3A4*1/*1B* | 4 | 4.3 |
| | *CYP3A4*1/*22* | 9 | 9.7 |
| *CYP3A5* | *CYP3A5*1/*3* | 8 | 8.6 |
| | *CYP3A5*3/*3* | 85 | 91.4 |
| CYP expression | | | |
| CYP3A4 | low expressers | 14 | 15.0 |
| | normal expressers | 77 | 82.8 |
| | high expressers | 2 | 2.2 |
| Co-medication | no | 15 | 16.1 |
| | clozapine | 30 | 32.2 |
| | haloperidol | 8 | 8.6 |
| | quetiapine | 12 | 12.9 |
| | risperidone | 16 | 17.2 |
| | clonazepam | 20 | 21.5 |
| | valproic acid | 11 | 11.8 |
| | lamotrigine | 3 | 3.2 |
| | propranolol | 21 | 22.6 |
| | metoprolol | 15 | 16.9 |
| | metformin | 10 | 10.8 |

*median (min; max); **_CYP2D6nf_: **3, *4, *5, *6* and **4xN*; ***_CYP2D6red_: **10* and **41*

Table 2. $K_i$ values (μM) for *in vitro* inhibition of aripiprazole metabolism by risperidone, 9-hydroxy-risperidone, propranolol and metoprolol*

| | Risperidone | 9-Hydroxy-risperidone | Propranolol | Metoprolol |
|---|---|---|---|---|
| Dehydroaripiprazole | 8.4±2.64 | 9.3±2.97 | 48.2±9.16 | 13.4±2.80 |
| Hydroxy-aripiprazole | 9.1±1.70 | 13.9±0.26 | - | - |
| *N*-dealkyl aripiprazole | 57.0±7.11 | - | - | - |

**In vitro* inhibition of aripiprazole metabolism was carried out at three different concentrations of aripiprazole (10, 25, 50 μM) and eight concentration points of risperidone, 9-hydroxy-risperidone, propranolol or metoprolol (1-50 μM) using hepatic microsomes of three tissue donors.

**Figure legends**

**Fig. 1**: The influence of the patients' *CYP2D6* genotypes (A) and CYP3A-status (*CYP3A5* genotype and CYP3A4 expression) (B) on aripiprazole plasma concentrations.

*nf*: non-functional *CYP2D6* variations (**3, *4, *5, *6* and **4×N*); *red*: reduced function variations (**10* and **41*); *×N*: allele duplication; △: *CYP3A5*1* carrier; *: $P<0.01$; **: $P<0.0001$

**Fig. 2:** The influence of the patients' *CYP2D6* genotypes on dehydroaripiprazole/aripiprazole concentration ratios.

*mut*: non-functional and reduced function *CYP2D6* alleles (**3, *4, *5, *6, *4×N, *10* and **41*); *×N*: allele duplication; *: $P<0.001$; **: $P<0.0001$

**Fig. 3**: PLS model for aripiprazole plasma concentration. (A) Principal component analysis with input variables of aripiprazole daily dose/patients' bodyweight, patients' CYP3A-status (CYP3A4 expression, *CYP3A5* genotype), *CYP2D6* genotype; (B) Aripiprazole plasma concentrations predicted by the PLS model.

To simplify the data for the PLS modelling, some of the *CYP2D6* genotype groups were combined and three *CYP2D6* genotype groups were created, *CYP2D6*1/*1* (for *CYP2D6*1/*1* and *CYP2D6*1/*1 ×N*), *CYP2D6*1/mut* (for *CYP2D6*1/nf* and *CYP2D6*1/red*) and *CYP2D6mut/mut* (for *CYP2D6nf/nf* and *CYP2D6nf/red*).

**Fig. 4**: The effect of co-medication with CYP2D6 inhibitors (risperidone, metoprolol or propranolol) on patients' aripiprazole plasma concentrations. Boxes with the line inside represent the range and median, whereas the whiskers are for minimum and maximum values. The number of patients (N) in each group is also indicated. In *CYP2D6*1/*1* and **1/*1 ×N* group, co-medication with CYP2D6 inhibitor risperidone, metoprolol and propranolol was for 7, 7 and 8; in *CYP2D6*1/mut* group, 7, 7 and 10; in *CYP2D6mut/mut* group, 2, 1 and 3, respectively.

*mut*: loss-of-function *CYP2D6* allele; *×N*: allele duplication; *: $P<0.05$; **: $P<0.0001$

Figure 1_revised

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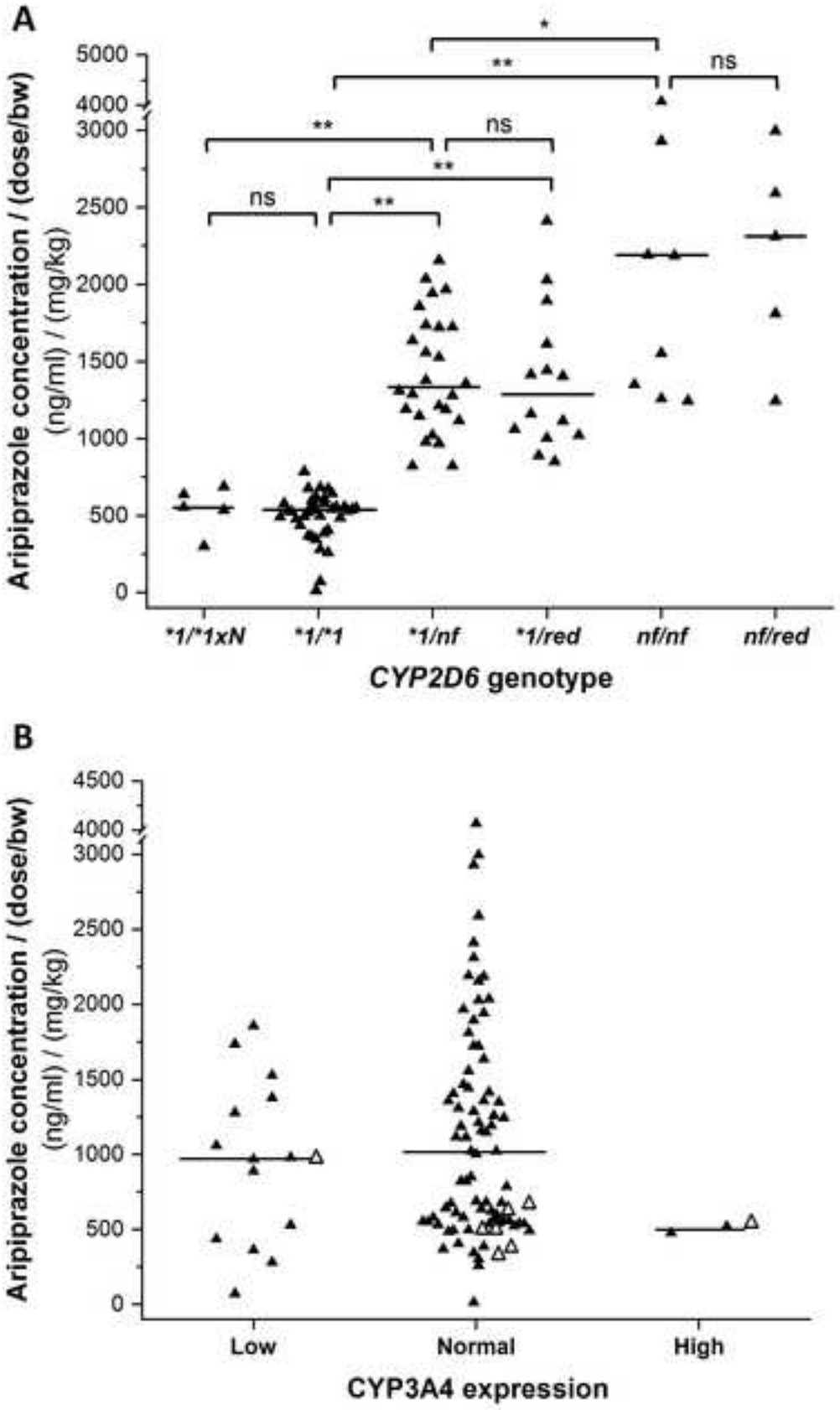


Figure 2



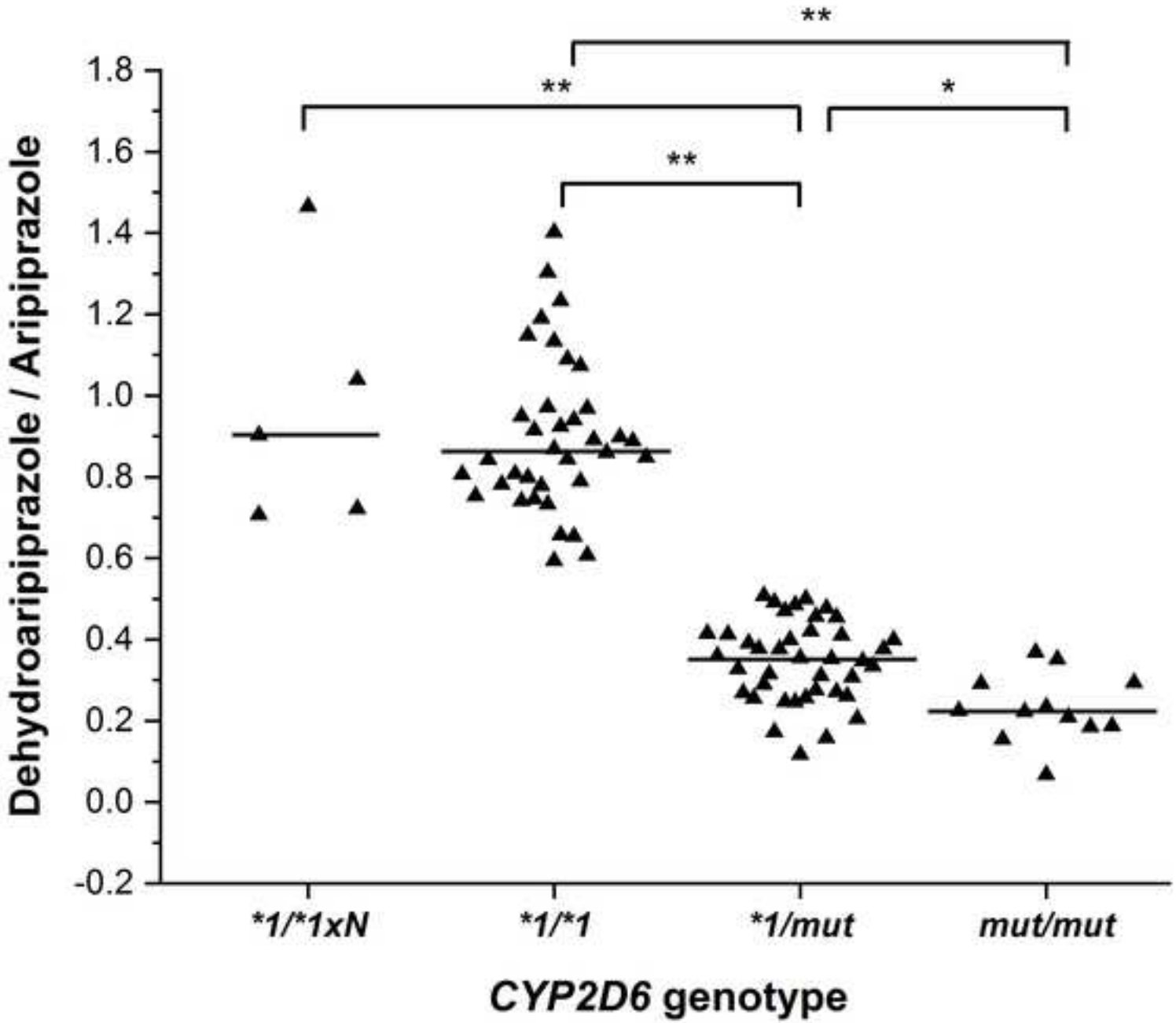
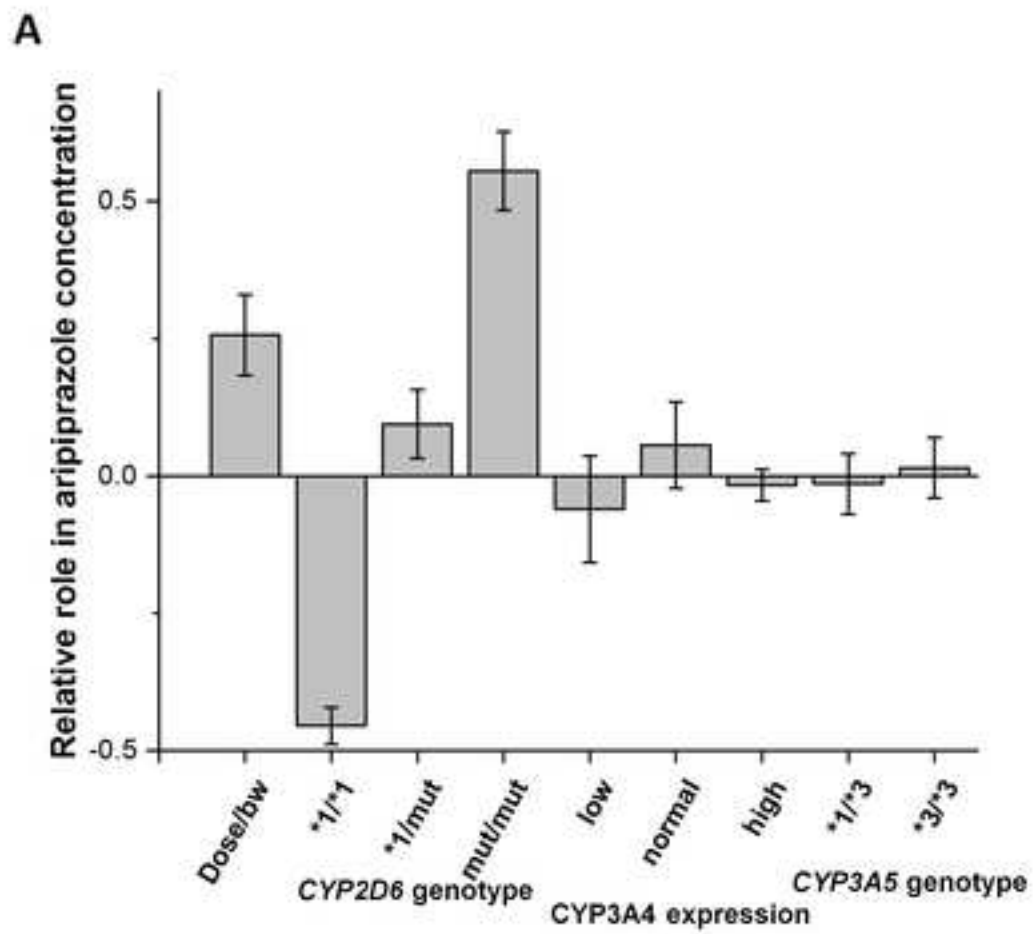


Figure 3





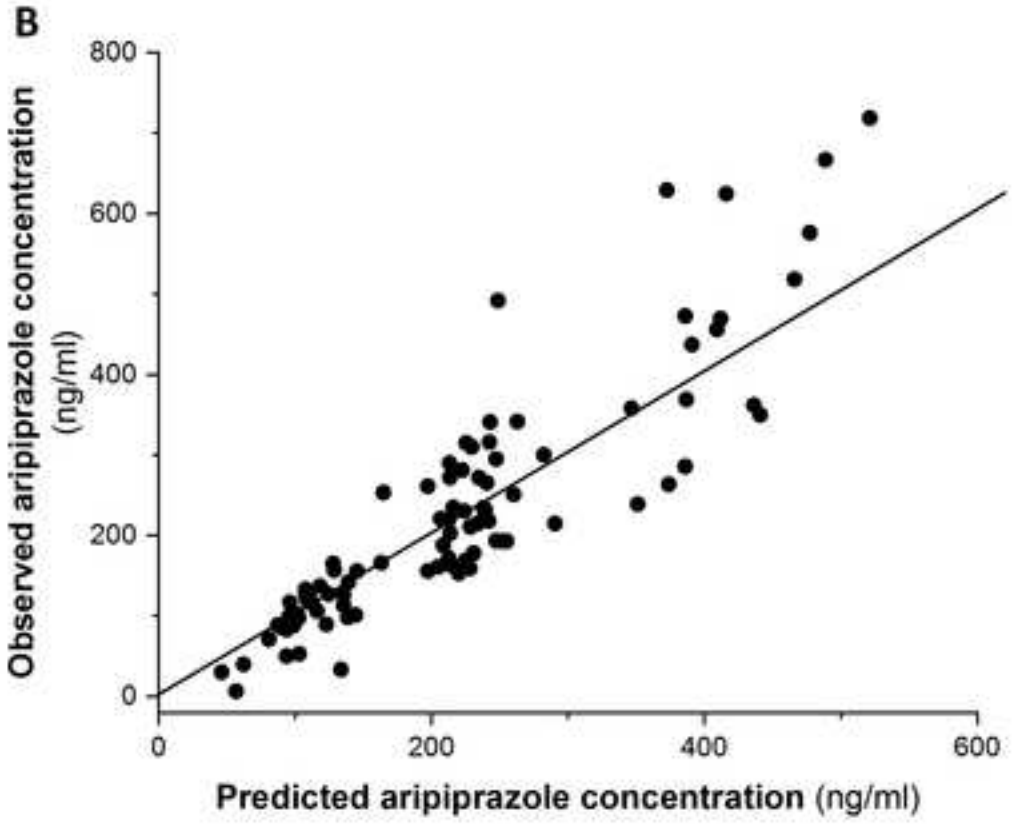



Figure 4_revised

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