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Effect of CYP2D6 and CYP2C19 genotypes on atomoxetine serum levels – a study based on therapeutic drug monitoring data

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Abstract

Atomoxetine is mainly metabolized by CYP2D6 while CYP2C19 plays a secondary role. It is known that patients carrying genotypes encoding decreased/absent CYP2D6 metabolism obtain higher atomoxetine concentrations and are at increased risk of adverse effects. Here, we aimed to investigate the added effects of reduced-function CYP2C19 genotype on atomoxetine concentrations in real-world settings. Serum atomoxetine concentrations and CYP2D6/2C19 genotypes were included from a therapeutic drug monitoring service. Patients were first subgrouped according to CYP2D6 encoding normal, reduced or absent CYP2D6 metabolism, referred to as normal (NM), intermediate (IM) or poor metabolizers (PM). Then, the effect of reduced-function CYP2C19 genotypes was investigated. Genotyping of the CYP2D6 nonfuctional or reduced variant alleles comprised CYP2D6*3-*5, *9-*10 and *41. For CYP2C19, the CYP2C19*2 was analysed to define metabolizer phenotype. Dose-adjusted serum atomoxetine concentration was the exposure measure. Using a patient cohort (n=315), it was found that CYP2D6 IM and PM patients had 1.9-fold (95%CI: 1.4-2.7) and 9.6-fold (5.9-16) higher exposure of atomoxetine compared with CYP2D6 NMs. CYP2C19*2 carriers had 1.5-fold (1.1-2.2) higher atomoxetine exposure than non-carriers regardless of CYP2D6 genotype. CYP2D6 genotype has a great impact on atomoxetine exposure, where our real-world data suggest atomoxetine dose requirements to be around half and one-tenth in CYP2D6 IM and PM vs. NM patients, respectively. When adding CYP2C19 genotype as a factor of relevance for personalized atomoxetine dosing, CYP2C19*2 carriers should further reduce the dose by a third. These findings suggest that pre-emptive CYP2D6/CYP2C19 genotyping should be performed to individualize atomoxetine dosing and prevent adverse effects.

Title page

Title

Effect of *CYP2D6* and *CYP2C19* genotypes on atomoxetine serum levels

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Running title

Atomoxetine and *CYP2D6/2C19* genotypes

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Principal Investigator statement

Espen Molden is the principal investigator

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What is already known about this subject?

Atomoxetine is mainly metabolized by CYP2D6 with a secondary involvement of CYP2C19.

Previous clinical studies have shown higher atomoxetine AUC, C_{max} and longer half-life in patients with the CYP2D6 poor metabolizer (PM) phenotype compared to normal metabolizers (NMs). The CPIC dosing guidelines advise reducing the dose by 50% in CYP2D6 PMs, while potential dose adjustments according to *CYP2C19*genotype is not mentioned.

What this study adds:

The present study shows that the differences in atomoxetine exposure (concentration dose^{-1} ratio) are almost 10-fold between CYP2D6 PMs and NMs, thus suggesting that the dose reduction in PMs should be substantially more than 50% as indicated by the CPIC guidelines.

*CYP2C19*2* allele carriers had on average 50% higher atomoxetine serum concentrations compared to non-carriers regardless of CYP2D6 genotype.

Abstract

Aim

Atomoxetine is mainly metabolized by CYP2D6 while CYP2C19 plays a secondary role. It is known that patients carrying genotypes encoding decreased/absent CYP2D6 metabolism obtain higher atomoxetine concentrations and are at increased risk of adverse effects. Here, we aimed to investigate the added effects of reduced-function*CYP2C19* genotype on atomoxetine concentrations in real-world settings.

Methods

Serum atomoxetine concentrations and *CYP2D6/2C19* genotypes were included from a therapeutic drug monitoring service. Patients were first subgrouped according to CYP2D6 encoding normal, reduced or absent CYP2D6 metabolism, referred to as normal (NM), intermediate (IM) or poor metabolizers (PM). Then, the effect of reduced-function *CYP2C19*genotypes was investigated. Genotyping of the CYP2D6 nonfuctional or reduced variant alleles comprised *CYP2D6*3 -*5 ,*9 -*10* and **41* . For CYP2C19, the *CYP2C19*2*was analysed to define metabolizer phenotype. Dose-adjusted serum atomoxetine concentration was the exposure measure.

Results

Using a patient cohort (n=315), it was found that CYP2D6 IM and PM patients had 1.9-fold (95%CI: 1.4-2.7) and 9.6-fold (5.9-16) higher exposure of atomoxetine compared with CYP2D6 NMs. *CYP2C19*2*carriers had 1.5-fold (1.1-2.2) higher atomoxetine exposure than non-carriers regardless of CYP2D6 genotype.

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Conclusion

CYP2D6 genotype has a great impact on atomoxetine exposure, where our real-world data suggest atomoxetine dose requirements to be around half and one-tenth in CYP2D6 IM and PM vs. NM patients, respectively. When adding CYP2C19 genotype as a factor of relevance for personalized atomoxetine dosing, *CYP2C19*2* carriers should further reduce the dose by a third. These findings suggest that pre-emptive CYP2D6/CYP2C19 genotyping should be performed to individualize atomoxetine dosing and prevent adverse effects.

Introduction

Attention deficit hyperactivity disorder (ADHD) has a reported prevalence of between 3-5% in children and adults, and is more common in males than females[1,2]. Typical symptoms include inattention, hyperactivity and impulsivity [3]. A number of young patients will continue to have symptoms as adults, although studies have shown that a proportion of patients diagnosed as adults did not have ADHD as children / adolescents[4]. Treatment with stimulants (primarily methylphenidate and amphetamine) is recommended as first-line therapy when medical treatment is indicated in children (over 6 years of age), adolescents and adults[2]. Stimulants affect the central nervous system by blocking presynaptic noradrenaline and dopamine reuptake in neuronal synapses. Atomoxetine is a second-line treatment alternative that acts primarily as a selective presynaptic noradrenaline-reuptake inhibitor, without a direct effect on presynaptic dopamine reuptake[5]. Microdialysis measurement in rats has shown increased extracellular levels of noradrenalin and dopamine in the prefrontal cortex in response to atomoxetine, but unaltered dopamine levels in the striatum and nucleus accumbens[6]. The result is that atomoxetine does not have the same abuse liability as the stimulants methylphenidate and amphetamine. Atomoxetine is recommended for ADHD-patients for whom stimulants are contraindicated or who have experienced significant adverse effects when using stimulants. However, cardiovascular adverse effects, including raised blood pressure and tachycardia may also occur when using atomoxetine[7].

Atomoxetine is metabolized to two metabolites – 4-hydroxyatomoxetine (and further to 4-hydroxyatomoxetine-O-glucuronide) and, to a lesser degree, *N* -desmethylatomoxetine[5]. Although 4-hydroxyatomoxetine exhibits potent inhibition of noradrenaline reuptake (as potent as atomoxetine itself), it is present in only small amounts (up to 1 % of the atomoxetine concentration) and, therefore, has only a minor contribution to the therapeutic effect of atomoxetine.*N* -desmethylatomoxetine is also unlikely to have an impact on treatment effect due to lower activity than the parent compound. Factors determining the metabolism and concentration levels of the parent compound are therefore crucial in determining response to atomoxetine treatment. The metabolism of atomoxetine to 4-hydroxyatomoxetine is catalysed primarily by the cytochrome P450-enzyme CYP2D6, while the metabolism of atomoxetine to *N* -desmethylatomoxetine is catalysed by CYP2C19[8]. Both CYP2D6 and CYP2C19 are highly polymorphic enzymes. Identification of genetic variants with increased, reduced or no enzymatic activity has been described. Among Caucasians, about 7% and 3-4% of the population, respectively, have variants with the CYP2D6 and CYP2C19 poor metabolizer (PM) genotype, while 1-2% and 4% have the CYP2D6 and CYP2C19 ultra-rapid (UM) genotype, respectively. Following metabolism, atomoxetine is primarily excreted in urine as glucuronidated metabolites[8].

Studies of atomoxetine pharmacokinetics have shown a significant association with CYP2D6 activity, finding a 5-time increase in maximum serum concentration (C_{max}) and a 10-time increase in cumulative serum concentration (area under the curve, AUC), increased total body clearance and extended elimination half-life (t 1/2) in patients having the CYP2D6 PM phenotype compared to normal metabolizers (NM)[9,10]. Similar effects of CYP2D6-phenotype were also observed in children and adolescents (7-14 years of age)[11]. Patients with PM phenotype may therefore have higher atomoxetine levels and a higher risk of adverse effects, since many adverse effects are dose dependent. Care is therefore advised when dosing atomoxetine in this patient group. The Clinical Pharmacogenetics Implementation Consortium (CPIC) have summarized treatment-recommendations for both children and adults according to CYP2D6 phenotype in international guidelines[12]. However, pre-emptive *CYP2D6* genotyping of patients starting atomoxetine is still not implemented in clinical practice. Data regarding the effect of *CYP2C19* genotype on atomoxetine metabolism,

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concentration and effect is more limited, although one study found an association between serum concentration and *CYP2C19*genotype in Asian patients[13].

The aim of the present study was to investigate the added effects of*CYP2C19* genotypes on atomoxetine concentration in patients with known *CYP2D6* genotype using therapeutic drug monitoring (TDM) data.

Methods

Subjects

The study was based on routine TDM data collected at the Center for Psychopharmacology, Diakonhjemmet Hospital (Oslo, Norway) over the period April 2005 - August 2021. Sample information was extracted from the standard TDM requisition forms, including concomitant medication, time interval between last dose and sampling, and daily dosage. The recommended sampling time interval for atomoxetine is 4-8 hours, in concordance with the Norwegian harmonization project for ADHD-drug laboratory analysis[14] and selected to coincide with primary therapeutic effect. The study criteria were serum samples of atomoxetine without (*i*) use of enzyme inducers (carbamazepine, phenytoin and phenobarbital) and/or (*ii*) use of CYP2D6 inhibitors (paroxetine, fluoxetine and bupropion) and (*iii)* available*CYP2D6* and *CYP2C19* genotype records. The outcome measures of interests were (*i*) dose-adjusted serum concentration (C D^{-1} ratio), (*ii*) unadjusted serum concentrations, (*iii*) daily dose, and (*iv*) risk of having undetectable serum concentration of atomoxetine.

The study was approved by the Regional Committee for Medical and Health Research Ethics (REK-235205), and did not require informed patient consent, as only historical, anonymized data were included without the potential to cause any harm.

Serum concentration analyses of atomoxetine

During the time course of the retrospective data collection, two slightly different, cross-validated routine LC-MS/MS methods were applied for atomoxetine analyses. For the most recent routine LC-MS/MS method, serum samples were prepared by protein precipitation in 96-deep well plates using a Microlab Star pipetting robot (Hamilton, Reno, NV, USA) in a semi-automated sample preparation procedure. The LC system was a Vanquish-UHPLC (Thermo Fisher Scientific, Waltham, MA, USA), and chromatographic separation was performed by an XBridge BEH C18-column (2.6 µm, 2.1x75 mm; Waters, Milford, MA, USA) using gradient elution at 35°C with a mix of ammonium acetate buffer (pH = 4.8) and acetonitrile (20-52%). The retention time was 2.08 min for atomoxetine. Detection used a QExactive Orbitrap mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA), operated in positive ionization mode acquiring full scan data at a resolution of 70,000 within the 100-1,500 Da scan range. Atomoxetine was quantified in full scan acquisition mode, while accurate data dependent MS2 analysis was simultaneously triggered to permit confirmation of its identification. The lower limit of quantification (LLOQ) was 5 nM. The precision and accuracy of atomoxetine concentrations were <5%.

CYP2D6 and CYP2C19 Genotyping

Genotyping of *CYP2D6* and *CYP2C19* variant alleles were performed using Taqman-based real-time PCR assays implemented and validated for routine pharmacogenetic testing at the Center for Psychopharmacology, Diakonhjemmet Hospital, Oslo, Norway, described in detail elsewhere[15,16].

The CYP2D6 pharmacogenetic panel included (*i*) Null alleles*CYP2D6*3* , **4* , **6* , (*ii*) reduced-function (Red) alleles **9* , **10* and **41* , as well as (*iii*) copy number analysis to identify *CYP2D6*5* (whole gene deletion) allele and duplication of functional alleles (*CYP2D6*1x3*). The CYP2C19 pharmacogenetic panel included the non-functional allele *CYP2C19*2.* For the purpose of the present study, *CYP2D6*1* and *CYP2C19*1* were assigned to the alleles where no variant allele was detected with the abovementioned procedure. Since the assays could not discriminate between multiplications of functional, non-functional or reduced-functional variant allele combinations of *CYP2D6* gene, patients with multiplied alleles of these combinations were not included. The patients were divided into the following CYP2D6 genotype-defined

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categories according to according to CPIC guidelines[17]: (*i*) poor metabolizers (PMs;*CYP2D6Null/Null*), (*ii*) intermediate metabolizers (IM;*CYP2D6Null/Red* , and *CYP2D6*1/Null* and*CYP2D6Red/Red*), (*iii*) normal metabolizers (NMs;*CYP2D6*1/Red* , and *CYP2D6*1/*1*), and (*iv*) ultrarapid metabolizers (UMs; *CYP2D6*1x3*). Patients were also categorized based on *CYP2C19*2* carriers or noncarriers.

Data analysis

As a measure of exposure, (*i*) C D^{-1} ratio of atomoxetine was defined as the main outcome measure of interest, as well as (*ii*) unadjusted serum concentration, (*iii*) daily dose, and (*iv*) risk of having undetectable serum concentration of atomoxetine. For outcome measures (*i-iii*), linear mixed model analyses (using random intercept and the restricted maximum likelihood model) were used to allow for inclusion of multiple samples per patient with age, sex and blood sampling time as covariate, if not otherwise described. The C D^{-1} ratios and absolute concentrations of atomoxetine were ln-transformed prior to analysis in order to ensure normal distribution of the data. Furthermore, several sensitivity analyses were performed to estimate the robustness when estimating the effects of *CYP2D6/2C19* genotype subgroups, including limiting inclusion of serum samples solely to those withdrawn 4-8 hours post dose. To calculate the risk of having undetectable serum samples, a mixed logistic regression model allowing inclusion of all serum samples per patients was used with risk of having undetectable serum samples as reference. The other proportions (i.e., male sex, samples with available sampling time, samples with dose, undetectable serum samples, patients with at least one undetectable serum sample and*CYP2D6/2C19* genotype subgroups) were compared using Fisher's exact test.

All statistical analyses were performed in SPSS?, version 25.0 (IBM? SPSS? Statistics, Armonk, NY, USA). GraphPad version 4 was used for graphical presentations (GraphPad Software, San Diego, CA). In figure 1, linear mixed model was used as described above but with no covariates and with Sidak-adjusted p-values. For the other analyses, the comparison-wise alpha was set at 0.05 and no multiplicity adjustments were made. The estimated means are given with either standard error (SE) or 95% confidence intervals (CI).

Results

In total, 315 patients (671 serum samples) aged from 6 to 73 years were included (table 1). The average age was 24.9 years (CI: 23.3, 26.4). Information on blood sampling time adhering to the recommended time interval (4-8 hrs) was available for 47% of the patients. For these patients, the average blood sampling time post-dose was 5.4 hrs (CI: 5.2, 5.6 hrs). Information about dose was available for 87% of the TDM samples. As many as 21.5% of the patients had recorded at least one undetectable serum sample. The proportions of CYP2D6 UMs, NMs, IMs and PMs were 1.2%, 52%, 37% and 10%, respectively, while the proportion of *CYP2C19*2* allele carriers was 35% (table 1).

Figure 1 shows the absolute serum concentration and daily dose of atomoxetine for patients subgrouped to the various *CYP2D6* and*CYP2C19* genotypes. The serum concentration of atomoxetine was systematically higher for PMs ($p<0.001$) and IMs ($p<0.048$) regardless of carrying the *CYP2C19*2* allele variant but were not significantly altered in UMs ($p>0.9$), when compared to NMs not carrying the *CYP2C19*2* allele variant (figure 1A). Despite this, insignificant differences in dosing were registered for PMs and IMs when compared to NMs or UMs (figure 1B; $p>0.05$).

In the studied population, a high variability of atomoxetine C D^{-1} ratio was observed (>85-fold difference; 10-90 percentile: 0.88-75 nM mg^{-1}). As CYP2D6 NMs and UMs had no statistical differences in absolute concentrations ($p=0.90$) and C D^{-1} ratio of atomoxetine ($p=0.77$), these groups were merged for the remaining analyses. Compared to CYP2D6 NM/UMs, a significantly increased atomoxetine C D^{-1} ratio was observed for CYP2D6 IMs and PMs (IM: +95%, $p<0.001$; PM: +863%, $p<0.001$; table 2), respectively. A similar effect was observed when CYP2D6 UMs were excluded (model 5, supplementary table 1). Moderately higher C D^{-1} ratio of atomoxetine was observed in*CYP2C19*2* allele carriers versus noncarriers (+54%, $p=0.011$; table 2). A statistically significant decrease in C D^{-1} ratio and increase in daily dose were observed with increasing age, respectively (C D^{-1} ratio: -0.020 nM/mg per year, $p<0.001$; Dose: +0.83 mg per year, $p<0.001$; table 2). Furthermore, significantly increased absolute atomoxetine concentrations were observed for CYP2D6 IMs

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and PMs when comparing to NM/UMs (IMs: +65%, p=0.006; PMs: +570%, p<0.001; table 2), respectively. Statistically higher absolute concentration of atomoxetine was observed for *CYP2C19*2* allele carriers versus non-carriers (+49%, p=0.022; table 2). Moreover, daily dose was statistically lower for CYP2D6 IM and PMs compared to NM/UMs (IMs: -16%, p=0.022; PMs: -22%, p=0.029), but not between *CYP2C19*2* allele carriers vs non-carriers (p=0.78; table 2). In addition, inclusion of interaction terms to the mixed model such as age x CYP2D6 genotype, age x *CYP2C19*2* allele carriers or CYP2D6 genotype x *CYP2C19*2* allele carriers turned out to be insignificant (model 1-4 supplementary table 1). Sensitivity analysis, only including blood samples taken 4-8 hrs post-dose, revealed similar quantitative effects on atomoxetine C D^{-1} ratio of the various *CYP2D6* genotype subgroups, *CYP2C19*2* allele carriers and age (model 6, supplementary table 1).

For CYP2D6 UMs (three out of four; 75%) and PMs (12.5%), the proportions of patients with at least one undetectable serum sample were higher and lower when compared to the other genotype subgroups (NMs, 26.4%; IMs, 15.5%; p=0.0071), respectively. In the mixed logistic regression model, including all serum samples, a lower odds ratio (OR) of having an undetectable serum sample was observed for CYP2D6 IMs (OR: 0.50, p=0.037) and PMs (OR: 0.34, p=0.068), compared to CYP2D6 UM/NMs (table 2). No effect on the risk of having an undetectable serum sample was observed between *CYP2C19*2* carriers vs non-carriers (p=0.12; table 2). Interestingly, increasing age was associated with an increased OR of undetectable concentrations by +3% per year (p=0.002; table 2).

Discussion

The present study showed that *CYP2D6* genotype has a great impact on atomoxetine exposure, where IM and PM patients have 1.9-fold and 9.6-fold higher serum concentrations of atomoxetine per mg dose compared to NMs/UMs. When adding CYP2C19 genotype as a factor of relevance for personalised atomoxetine dosing, *CYP2C19*2* carriers had 1.5-fold higher serum concentrations of atomoxetine per mg dose than non-carriers regardless of CYP2D6 genotype. CYP2D6 PMs and IMs carrying the *CYP2C19*2* allele should have doses equivalent to [?]10% and 35% of the normal starting dose, respectively, to target the same exposure as CYP2D6 NMs not carrying the *CYP2C19*2* allele. These findings suggest that pre-emptive genotyping of CYP2D6/CYP2C19 should be performed to individualise atomoxetine dosing and minimise the risk for adverse effects.

According to CPIC dosing recommendations for atomoxetine[12], dosing for CYP2D6 PMs should be reduced to approximately half of the approved dose in the product label information. Our study, however, suggests that an even lower dose (i.e., [?]10% of the starting dose) should be used in CYP2D6 PMs to avoid potential dose-dependent adverse effects. In practice, starting dose selection will be limited by available formulation and lowest available dose, with only oral solution forms suitable for the lowest doses. Further, our study suggests that for CYP2D6 IMs, a dose reduction equivalent to 50% of the recommended dose should be considered to target the same drug exposure as NMs, consistent with results from a previous study[18]. An even larger dose-reduction is warranted if the CYP2D6 PMs and IMs are carrying the *CYP2C19*2*allele (dose reduction of [?]90% and 65%, respectively), compared to CYP2D6 NMs not carrying the *CYP2C19*2* allele. There was no significant interaction effect between the various CYP2D6 genotype subgroups and *CYP2C19*2* allele carriers (p[?]0.13), thus, indicating an additive effect on atomoxetine exposure of*CYP2C19*2* allele variant carriers regardless of CYP2D6 genotype. In addition, our study shows that the quantitative differences in atomoxetine exposure between the various *CYP2C19* and*CYP2D6* genotypes were similar regardless of age. In patients who are CYP2D6 UM, very limited data exists, but it is unlikely these patients would achieve adequate serum concentrations with standard atomoxetine dosing[12,19]. Indeed, we showed that CYP2D6 UMs had a higher probability of having at least one undetectable atomoxetine concentration (three out of four patients) compared to all other *CYP2D6* genotypes, which indicates higher risk of undetectable levels and therapeutic failure. However, due to the low numbers of CYP2D6 UMs in the present study, no statistically significant differences were observed between NMs and UMs for C D^{-1} ratio, absolute concentrations and daily dose of atomoxetine, as well as risk of undetectable samples in the mixed model analyses. The CPIC's recommendations are based on studies showing superior treatment response

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and compliance in CYP2D6 PMs versus NMs. It is therefore likely that the CYP2D6 PMs in the present study may have experienced improved therapeutic response but also increased dose-dependent side effects compared to the other *CYP2D6* genotype subgroups. It is possible that this superior treatment response also resulted in better treatment adherence and fewer undetectable atomoxetine samples. Unfortunately, clinical data on therapeutic response were unavailable. A study based on pooled data from clinical trials conducted by Michelson et al.[20] found that PMs showed a greater clinical improvement, measured by ADHDRS-score, and lower treatment drop-out than patients with normal CYP2D6 activity. Interestingly, but unsurprisingly, these patients also had higher serum levels of atomoxetine (C_{max} sampled 1-hour post-dose, PMs vs NMs: approx. 3300 vs 650 nM on the same dose) and risk of adverse effects, including raised pulse and diastolic blood pressure, tremor, decreased appetite and insomnia. Trzepacz, PT *et al.* found a significant difference in weight, BMI and pulse in two open-label phase 3 studies but, conversely, no differences in clinical effect were observed between the *CYP2D6* genotype subgroups[9]. Fijal, BJ *et al.* studied the effect of CYP2D6 activity and safety associated with atomoxetine treatment in adult patients over a period of 25 weeks at multiple international centers[21]. In addition to comparing NM and PM phenotypes, as in earlier studies, they distinguished between patients with UM and IM phenotypes. The study found an increased risk of adverse effects in patients with poor CYP2D6 activity, including reduced appetite, dry mouth, erectile dysfunction, hyperhidrosis, insomnia and urinary retention. Diastolic blood pressure and pulse were also significantly higher, while BMI was significantly lower. Patients with IM also had a higher risk of dry mouth and insomnia. Therefore, it is possible that the higher burden of adverse effects observed in CYP2D6 PMs may outweigh the potential improvement in therapeutic response compared to NMs.

A strength of the present study was the relatively large dataset, of not only adult users, but also children and adolescents that are previously less studied. We also had the possibility to correct the results for blood sampling time (trough concentrations, blood sampling 4-8 hrs post-dose). One obvious weakness of the present study was the lack of clinical information about each patient, including therapeutic effectiveness and treatment outcome, information on renal and hepatic status (e.g., Child-Pugh B and C category), as well as existing somatic comorbidities that would be of value. Concentrations are not corrected for patient weight as this data was not available. Patient weight may play a role in atomoxetine pharmacokinetics, especially in younger patients where weight-corrected dosing is recommended (i.e., patients under 70 kg in weight). Therefore, the patients' age was included in the statistical models as age may account for weight indirectly. In addition, dose administration intervals were not known and may generate some noise in the estimates but are unlikely skewed between the various *CYP2D6* genotype subgroups and *CYP2C19*2* allele carriers versus noncarriers. Information about steady-state could not be confirmed but steady-state is a prerequisite for TDM, and this is clearly communicated to doctors requesting TDM. It is therefore likely that the great majority of atomoxetine serum samples are at steady-state. A higher proportion of males (59%) was observed in the present study population which reflects the higher proportion of males diagnosed with ADHD as reported elsewhere[1,2]. Thus, the study population is likely representative and comparable to other studies.

In conclusion, both *CYP2D6* and *CYP2C19* genotypes have an impact on atomoxetine exposure regardless of age, where our real-world data suggest atomoxetine dose requirements to be equivalent to [?]10% and 35% of the starting dose in CYP2D6 IM and PM patients carrying the *CYP2C19*2* allele variant versus CYP2D6 NM patients not carrying the *CYP2C19*2* allele variant, respectively, to target the same atomoxetine exposure. These findings suggest that pre-emptive genotyping of *CYP2D6* / *CYP2C19* should be performed to individualize atomoxetine dosing and prevent adverse effects.

Conflict of interest statement

The authors declare no conflict of interest.

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Data availability statement

The data that supports the findings of this study are available upon reasonable request to the corresponding author. The data are not publicly available due to privacy and ethical restrictions.

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Tables

Table 1. Demographics of the study population.

Variables	**All**
	Value
Females /males, n (TDM samples)	130 (272) / 185 (399)
Age, years, mean (95%CI)[a]	24.9 (23.3, 26.4)
Prop. of samples with sampling time, n (n patients)	232 (148)
Sampling time, hours, mean (95%CI)[a]	5.4 (5.2, 5.6)
Prop. of samples with dose, n (n patients)	587 (274)
Dose, mg, mean (95%CI)[a]	53.4 (49.7, 57.2)
Prop. of undetectable serum samples (n patients)	80 (68)
Atomoxetine serum conc., nM, mean (95%CI)[a]	1385 (1194, 1577)
CYP2D6 genotype subgroups, n UM / NM / IM / PM	4 / 163 / 116 / 32
*CYP2C19*2* carriers / non-carriers	109 / 206
[a]The means were calculated with linear mixed model with no covariates. TDM, therapeutic drug monitoring; Sampling time, time between last dose and blood sampling; CYP, cytochrome P450; UM, ultra-rapid metabolizers; NM, normal metabolizers; IM, intermediate metabolizers; PM, poor metabolizers; CI, confidence intervals.	[a]The means were calculated with linear mixed model with no covariates. TDM, therapeutic drug monitoring; Sampling time, time between last dose and blood sampling; CYP, cytochrome P450; UM, ultra-rapid metabolizers; NM, normal metabolizers; IM, intermediate metabolizers; PM, poor metabolizers; CI, confidence intervals.

Table 2. Effects of CYP2D6/2C19 genotype subgroups, age and sex on dose-adjusted serum atomoxetine concentration (C D^{-1} ratio).

Variables	N (samples)	Absolute concentration#	Absolute concentration#	Absolute concentration#	Daily dose#	Daily dose	Daily dose	C:D ratio#	C:D ratio#	C:D ratio#	Risk of undetectable serum conc.	P c u d t s c
		Average (CI)*	Beta (SE)	p	Average (CI)*	Beta (SE)	p	Average (CI)*	Beta (SE)	p	OR (CI)	C (
Intercept			6.0 (0.17)	<0.001	39 (4.1)	<0.001			2.36 (0.18)	<0.001	0.071	- (
CYP2D6 UM/NM	133 (276)	6.0 (5.8, 6.3)	0 (0) 0.45 (0.16)	- 0.006	58 (53, 63)	0 (0) -8.8 (3.8)	- 0.022	2.11 (1.9, 2.3)	0 (0) 0.66 (0.17)	- <0.001	0 (0) 0.50 (0.26, 0.96)	0 - (
CYP2D6 IM	99 (204)	6.5 (6.2, 6.7)	<0.001 1.9 (0.24)	<0.001	49 (44, 55)	0.029 -13 (5.9)	0.029	2.77 (2.5, 3.0)	<0.001 2.26 (0.26)	<0.001	- 0.34 (0.10, 1.1)	- (
CYP2D6 PM	27 (66)	7.9 (7.5, 8.4)			45 (35, 56)			4.37 (3.9, 4.8)				
CYP2C19 non-carriers	173 (390)	6.6 (6.4, 6.8)	0 (0) 0.37 (0.16)	- 0.022	51 (47, 56)	0 (0) -1.1 (3.8)	- 0.78	2.87 (2.7, 3.1)	0 (0) 0.44 (0.17)	- 0.011	0 (0) 1.6 (0.88, 3.0)	0 0 (
*CYP2C19** carriers	86 (256)	7.0 (6.7, 7.3)			50 (44, 57)			3.30 (3.0, 3.6)				
Age (per year)			-0.005 (0.005)	0.31		0.83 (0.13)	<0.001		-0.020 (0.006)	<0.001	1.0 (1.0, 1.1)	0 (
Pseudo R^2 (marginal/ conditional)		0.167 / 0.401	0.167 / 0.401	0.167 / 0.401	0.163 / 0.618	0.163 / 0.618	0.163 / 0.618	0.248 / 0.526	0.248 / 0.526	0.248 / 0.526		

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Variables	N (samples)	Absolute concentration	Absolute concentration#	Absolute concentration#	Daily dose#	Daily dose	Daily dose	C:D ratio#	C:D ratio#	C:D ratio#	Risk of undetectable serum conc.	[illegible]
#The values are presented as ln-transformed values to ensure normal distribution. The average values were calculated with linear mixed model with age as covariate (the value of age is shown as *23.4 years). CYP, cytochrome P450; UM	#The values are presented as ln-transformed values to ensure normal distribution. The average values were calculated with linear mixed model with age as covariate (the value of age is shown as *23.4 years). CYP, cytochrome P450; UM	#The values are presented as ln-transformed values to ensure normal distribution. The average values were calculated with linear mixed model with age as covariate (the value of age is shown as *23.4 years). CYP, cytochrome P450; UM	#The values are presented as ln-transformed values to ensure normal distribution. The average values were calculated with linear mixed model with age as covariate (the value of age is shown as *23.4 years). CYP, cytochrome P450; UM	#The values are presented as ln-transformed values to ensure normal distribution. The average values were calculated with linear mixed model with age as covariate (the value of age is shown as *23.4 years). CYP, cytochrome P450; UM	#The values are presented as ln-transformed values to ensure normal distribution. The average values were calculated with linear mixed model with age as covariate (the value of age is shown as *23.4 years). CYP, cytochrome P450; UM	#The values are presented as ln-transformed values to ensure normal distribution. The average values were calculated with linear mixed model with age as covariate (the value of age is shown as *23.4 years). CYP, cytochrome P450; UM	#The values are presented as ln-transformed values to ensure normal distribution. The average values were calculated with linear mixed model with age as covariate (the value of age is shown as *23.4 years). CYP, cytochrome P450; UM	#The values are presented as ln-transformed values to ensure normal distribution. The average values were calculated with linear mixed model with age as covariate (the value of age is shown as *23.4 years). CYP, cytochrome P450; UM	#The values are presented as ln-transformed values to ensure normal distribution. The average values were calculated with linear mixed model with age as covariate (the value of age is shown as *23.4 years). CYP, cytochrome P450; UM	#The values are presented as ln-transformed values to ensure normal distribution. The average values were calculated with linear mixed model with age as covariate (the value of age is shown as *23.4 years). CYP, cytochrome P450; UM	#The values are presented as ln-transformed values to ensure normal distribution. The average values were calculated with linear mixed model with age as covariate (the value of age is shown as *23.4 years). CYP, cytochrome P450; UM	[illegible]

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Variables	N (samples)	Absolute concentration#	Absolute concentration#	Absolute concentration#	Daily dose#	Daily dose	Daily dose	C:D ratio#	C:D ratio#	C:D ratio#	Risk of un-de-tectable serum conc.	P c u d t s c

Figure legends

Figure 1. Inter- and intraindividual variability of serum concentrations and daily dose of atomoxetine in various CYP2D6/2C19 genotype subgroups. **A and B:** The effect of CYP2D6 genotype subgroups not carrying (*N*) or carrying the *CYP2C19*2* allele variant (**2*) on serum concentration (A) and daily dose (B) of atomoxetine. This effect was analysed using linear mixed model without covariates with CYP2D6 NM not carrying *CYP2C19*2* allele variant as reference group. *P-values <0.05, and the p-values are Sidak-adjusted. The various *CYP2D6* genotype subgroups are represented with red dots for poor metabolizers (PM), yellow dots for intermediate metabolizers (IM), green dots for normal metabolizers (NM) and blue dots for ultra-rapid metabolizers (UM), respectively.