

ARTICLE

Impact of the novel *CYP2C:TG* haplotype and *CYP2B6* variants on sertraline exposure in a large patient population

Line Skute Bråten^{1,2}  | Magnus Ingelman-Sundberg³ | Marin M. Jukic^{3,4} |
Espen Molden^{1,5} | Marianne Kristiansen Kringen^{1,2}

¹Center for Psychopharmacology, Diakonhjemmet Hospital, Oslo, Norway

²Department of Health Sciences, OsloMet – Oslo Metropolitan University, Oslo, Norway

³Section of Pharmacogenetics, Department of Physiology and Pharmacology, Biomedicum 5B, Karolinska Institutet, Stockholm, Sweden

⁴Department of Physiology, Faculty of Pharmacy, University of Belgrade, Belgrade, Serbia

⁵Department of Pharmaceutical Biosciences, School of Pharmacy, University of Oslo, Oslo, Norway

Correspondence

Line Skute Bråten, Center for Psychopharmacology, Diakonhjemmet Hospital, Forskningsveien 7, Pb 23 Vinderen, 0319, Oslo, Norway.
Email: line.braten@diakonpsyk.no

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Abstract

Sertraline is a commonly used SSRI antidepressant drug, metabolized by *CYP2C19* and *CYP2B6*, that exhibits a substantial interindividual variation in clinical response, of which only a part can be attributed to known genetic variants. In the current study we have examined the role of a newly discovered ultrarapid *CYP2C:TG* haplotype and *CYP2B6* variants in order to identify the possible missing heritability for such variation in sertraline response in a large patient population ($n = 840$). Compared to the reference group (*CYP2C19**1/*1, $n = 160$), sertraline exposure was increased by 128% in *CYP2C19* PMs ($n = 29$, $p < 0.001$) and decreased by about 20% in *CYP2C19* ultrarapid metabolizers (UMs) (homozygous carriers of *CYP2C19**17 and/or *CYP2C:TG* haplotype) with the diplotypes *CYP2C19**17/*17, *CYP2C:TG*/*TG*, or *CYP2C19**17/*CYP2C:TG* ($n = 135$, $p < 0.003$, $p = 0.022$, $p < 0.003$, respectively). Interestingly, in patients carrying the increased function *CYP2B6**4 allele, and also carrying the *CYP2C19**17 and *CYP2C:TG* alleles ($n = 10$), sertraline exposure was 35.4% lower compared to the reference group, whereas in subjects being poor metabolizers (PM) in both the *CYP2C19* and *CYP2B6* gene, the sertraline concentrations were raised by 189%. In summary, the *CYP2C19* variants including the *CYP2C:TG* haplotype had a significant impact on sertraline metabolism, as well as the *CYP2B6**4, *6, and *9 alleles. Knowing the *CYP2B6* and *CYP2C19* genotype, including the *CYP2C:TG* haplotype status, can prospectively be useful to clinicians in making more appropriate sertraline dosing decisions.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

Sertraline is a commonly used antidepressant subjected to metabolism by multiple enzymes with *CYP2C19* playing a key role along with *CYP2B6* and *CYP3A4*. Previous pharmacogenetic studies have reported significant effects of *CYP2C19* and *CYP2B6* genotypes on sertraline concentration, but with inconsistent findings probably reflecting that the concurrent effects of the genotypes have not

been investigated in a large patient population. Furthermore, the studies have not accounted for the recently discovered *CYP2C:TG* haplotype associated with increased *CYP2C19*-mediated metabolism.

WHAT QUESTION DID THIS STUDY ADDRESS?

Our study investigated the impact of the novel *CYP2C:TG* haplotype on sertraline serum concentration in a large population genotyped for *CYP2C19* (variants *2, *3, *4, and *17) and *CYP2B6* (variants *4, *6, and *9).

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

This study demonstrates that the novel *CYP2C* haplotype (*CYP2C:TG*) is associated with increased rate of *CYP2C19* metabolism of sertraline, as previously shown for escitalopram. In addition, patients carrying both the *CYP2B6*4* variant and *CYP2C19* genotypes encoding ultrarapid *CYP2C19* metabolism are at increased risk of underexposure and therapeutic failure when treated with standard recommended doses of sertraline, whereas patients with only inactive or decreased function alleles of both *CYP2C19* and *CYP2B6* have a lower capacity for sertraline metabolism than *CYP2C19* poor metabolizers carrying functional *CYP2B6* alleles.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

Preemptive genotyping of sertraline patients would include *CYP2C:TG*, *CYP2C19*, and *CYP2B6* variants to increase the dose precision of the drug in patients suffering from depression and/or anxiety.

INTRODUCTION

Sertraline is a selective serotonin reuptake inhibitor (SSRI), used in the treatment of major depressive, obsessive-compulsive, panic, post-traumatic stress, and social anxiety disorders.¹ There is an extensive interindividual variability in the pharmacokinetics of sertraline. The individual variability in sertraline pharmacokinetics could relate to differences in activity of the drug-metabolizing enzymes involved in sertraline metabolism, where the polymorphic enzymes *CYP2C19* and *CYP2B6* are of predominant importance; however, sertraline is also a substrate of *CYP2C9*, *CYP2D6*, and *CYP3A4*.^{2,3} Significant effects of *CYP2C19* variants on sertraline metabolism have already been observed by several studies, whereas there are no studies of *CYP2B6* and sertraline in a large patient population.²⁻⁶ By contrast, *CYP2D6* and *CYP2C9* variants are not predictors of sertraline metabolism, as shown by Braten et al.⁵ Regarding *CYP3A4*, a study with grapefruit juice,⁷ which is a potent inhibitor, reported a 1.5-fold increase in sertraline exposure during juice consumption, suggesting a relevant impact of *CYP3A4* on sertraline metabolism alongside *CYP2C19* and *CYP2B6*.⁷

CYP2C19 and *CYP2B6* are highly polymorphic which influences the metabolism of clinically relevant drugs. The major *CYP2C19* variants are the non-functional alleles *CYP2C19*2*, *3, *4, and *CYP2C19*17*, which

causes increased enzyme expression.⁸⁻¹² Among important *CYP2B6* allelic variants are *CYP2B6*6* (rs3745274, c.516G>T and rs2279343, c.785A>G) and *CYP2B6*9* (rs3745274, c.516G>T) associated with decreased enzyme activity and increased exposure of different *CYP2B6* substrates,¹³⁻¹⁵ while the *CYP2B6*4* variant (rs2279343, c.785A>G) has been associated with increased enzyme activity primarily based on in vitro studies.¹⁶ Currently, homozygous carriers of *CYP2B6*6* and/or *9 are classified by the CPIC guidelines as PMs, while homozygous carriers of the *CYP2B6*4* allele are assigned as UMs by PharmGKB and the CPIC guidelines.¹⁶⁻¹⁸

A previous study from our research group on 1200 Scandinavian patients demonstrated a significant increase in sertraline concentration in *CYP2C19* intermediate metabolizers (IMs) and poor metabolizers (PMs).⁵ In that study, however, only a marginally decreased serum concentration of sertraline was observed in *CYP2C19* ultrarapid metabolizers (UMs) (patients homozygous for *CYP2C19*17*) in comparison to normal metabolizers (NMs). Interestingly, a large proportion of the patients defined as *CYP2C19* NMs was underexposed to sertraline and required doses above the recommended 50 mg/day, potentially indicating the presence of unknown genetic variants causing increased sertraline metabolism among NMs.

Recently, we discovered a novel common *CYP2C*-haplotype defined by the co-occurrence of rs2860840T

and rs11188059G (*CYP2C:TG*), which was significantly associated with reduced exposure of the *CYP2C19* substrate escitalopram.¹⁹ In patients, the presence of the *CYP2C:TG* haplotype was associated with even lower escitalopram levels as compared to subjects carrying the *CYP2C19*17* allele. In the present study we aimed to examine the genetic basis for prediction of sertraline metabolism by investigating both the novel *CYP2C:TG* haplotype and *CYP2B6* alleles in a large patient population.

METHODS

The study encompassed patients who had been CYP-genotyped between January 1, 2017 and December 31, 2020 and for which the therapeutic drug monitoring (TDM) database at the Center for Psychopharmacology, Diakonhjemmet Hospital contained data on serum concentration of sertraline and metabolites. The patient population included from the TDM database represents individuals treated in primary practice and secondary health care units from all over Norway. The specific diagnoses of the patients are unknown.

The included patients had at least one sertraline concentration measurement at steady state (trough level), i.e., ≥ 4 days/dose intervals, with blood sample collection 10–30 h after the last dose intake. Exclusion criteria were: (i) comedication with the potent CYP inducers phenobarbital, phenytoin, and carbamazepine; (ii) comedication with the *CYP2C19* inhibitors omeprazole, esomeprazole, lansoprazole, pantoprazole, fluoxetine, and fluvoxamine; (iii) measured serum concentration of sertraline and/or the metabolite *N*-desmethylsertraline below the lower limit of quantification; and (iv) patient aged below 18 or above 90 years.

Information about sampling time and prescribed daily dose, time of last dose intake, as well as comedications, were retrieved from the requisition forms. The information about comedicated drugs was limited to the list written by the physician on the TDM requisition form, which is usually complete for psychotropic and other centrally acting central nervous system (CNS) drugs, while information on somatic drugs may be incomplete.

The study was approved by the Norwegian Regional Committee for Medical and Health Research Ethics (#2018/1848) and the Investigational Review board at Diakonhjemmet Hospital. All participants selected from the TDM database at the Center for Psychopharmacology were informed about the project and their right to withdraw from the study and the use of their biobanked blood sample for research purposes.

Serum concentration analysis of sertraline

Serum concentration analysis of sertraline was carried out at the Center for Psychopharmacology. In addition, determination of *N*-desmethylsertraline concentration is part of the routine TDM assay. However, as this metabolite is mainly formed by *CYP3A4*,⁷ and to a limited extent by *CYP2C19*,⁵ *N*-desmethylsertraline concentrations were not focused on in the present study.

During the inclusion period two different liquid chromatography–mass spectrometry (LC–MS) methods were used for routine TDM analysis. The methods were similar in terms of sample preparation (protein precipitation), chromatographic conditions, and type of internal standard. The most recent method was based on ultrahigh-performance LC (UHPLC) with a high-resolution accurate mass (HRAM) spectrometry system.

Briefly, serum samples were purified by protein precipitation mixing 200 μ l serum with 400 μ l acetonitrile–methanol (90/10 vol/vol), which included the internal standard (13C6-sertraline and 13C6-desmethylsertraline), followed by centrifugation for 10 min (3100g at 4°C). Subsequently, 4 μ l of purified sample were then injected into a Vanquish Binary UHPLC system coupled to a Q Exactive Orbitrap HRAM MS with electrospray ionization operated in positive ionization mode (Thermo Scientific, Waltham, MA, USA). Chromatographic separation was performed on a XBridge BEH C18 column (2.5 μ m, 2.1 \times 75 mm; Waters). The mobile phase gradient comprised a mixture of acetonitrile and ammonium acetate buffer (pH 4.8). Limits of detection for sertraline and *N*-desmethylsertraline were 4 and 8 nmol/L, respectively.

TaqMan real-time PCR analysis

Genotyping of *CYP2C19* had previously been performed with TaqMan genotyping assays (Thermo Fisher Scientific) using real-time polymerase chain reaction (PCR) implemented for routine pharmacogenetic analysis at the Center for Psychopharmacology. In addition to *CYP2C19* genotypes, *CYP2C9* and *CYP2D6* genotypes were also available in the routine laboratory database for the majority of the patients, as these genes are usually ordered together by the physicians.

The panel for *CYP2C19* genotyping included the non-functional alleles *CYP2C19*2* (rs4244285), *CYP2C19*3* (rs4986893), and *CYP2C19*4* (rs28399504), and the increased function allele *CYP2C19*17* (rs12248560). Routine genotyping of *CYP2C9* included the decreased function alleles *CYP2C9*2* (rs1799853) and *CYP2C9*3* (rs1057910), while routine *CYP2D6* genotyping included

the no function alleles *CYP2D6**3 (rs35742686), *CYP2D6**4 (rs3892097), *CYP2D6**5 (gene deletion), and *CYP2D6**6 (rs5030655), the decreased function alleles *CYP2D6**9 (rs5030656), *CYP2D6**10 (rs1065852), and *CYP2D6**41 (rs28371725), as well as copy number analysis to identify multiplication of the *CYP2D6* gene giving rise to ultrarapid metabolism.

Genotyping of the novel *CYP2C*-haplotype (rs2860840T and rs11188059G) was performed using predesigned TaqMan-based real-time PCR assays (Thermo Fisher Scientific).

Genotyping of *CYP2B6* included two *CYP2B6* variants rs3745274, c.516G>T and rs2279343, c.785A>G determining three different haplotypes: *CYP2B6**4 (c.516G+c.785G), *CYP2B6**6 (c.516T+c.785G), and *CYP2B6**9 (c.516T+c.785A). Absence of any of these two variants was assigned as *CYP2B6**1 (c.516G+c.785A).

Statistics

Impact of the tested *CYP2C19* and *CYP2B6* genetic variants on sertraline serum concentration was investigated using a multivariate-linear mixed model. First, the different genotypes of *CYP2C19* and *CYP2B6* were investigated for their quantitative impact on sertraline serum concentration. Second, patients were divided into genotype-predicted phenotype subgroups and the impact of the respective phenotype subgroups on sertraline serum concentration were investigated. To account for the variable number of serum concentration measurements per patient, the quantitative effects of the genotypes and genotype predicted phenotypes were estimated using multivariate linear mixed-model analysis, as this allows for inclusion of multiple measurements per patient. In the two mixed-model analyses, dose-adjusted serum concentrations of sertraline were used as the dependent variable, with the patient as the mixed effect (random) variable and genotype, sex, and time between last dose administration and blood sampling as fixed effect variables. When accounting for covariates, the mixed-model analysis estimated the effects of having any other genotype or phenotype than *1/*1 or NM, respectively. Covariates that differed significantly between the genotype subgroups were added to the mixed-model analysis if the significance reached $p < 0.05$. Samples with a harmonized serum concentration of sertraline outside the 95% percentile were not included in the analysis.

To restore normality of dose-adjusted serum concentrations of sertraline, these measurements were ln-transformed prior to linear mixed-model analysis. The natural logarithm of the normalized sertraline concentration levels (c) was used because of its linear dependence on the time between the drug intake and blood sampling

(t) and the elimination rate constant (K_e) according to the equation $\Delta \ln(c) = K_e t$.

In the mixed-model analysis of genotype-predicted *CYP2B6* phenotype subgroups, homozygote and heterozygote carriers of *CYP2B6**4 were categorized as ultrarapid metabolizers (UMs), non-carriers (*CYP2B6**1/*1) were set as normal metabolizer (NM), heterozygote carriers of *CYP2B6**6 or *9, in combination with *1 or *4, were set as intermediate metabolizers (IM), and homozygote carriers of *CYP2B6**6 or *9 as poor metabolizers (PMs). For *CYP2C19*, heterozygote carriers of non-functional alleles were defined as intermediate metabolizers (IMs), while homozygote carriers of non-functional alleles were defined as PMs. Since the novel *CYP2C:TG*-haplotype was associated with increased metabolism of the *CYP2C19* substrate escitalopram to a similar extent as *CYP2C19**17,¹⁹ carriers of *CYP2C19**17 and *CYP2C:TG*, in any combination, were defined as UMs. Patients carrying *CYP2C19**17 or *CYP2C:TG* in combination with *CYP2C19**1 were defined as rapid metabolizers (RMs) and *CYP2C19**1/*1 as NMs. Genotypes and their assigned phenotype subgroup are provided in Table 1.

Mixed-model estimates are presented as fold-changes in sertraline concentration (nM per 100 mg/day), which appear when back-transforming the beta values for each of the fixed effects (genotypes). After estimating the effects of the various genotypes, these were applied to predict the mean values of the dose-adjusted serum concentration for each genotype, using the mixed-model algorithm adjusted by mean values of the covariates (sampling time set to 20 h and age <65 years).

Statistical analysis was performed using R, version 4.0.3 and RStudio.^{20,21} A p -value of 0.05 was considered to be statistically significant.

RESULTS

In total, 840 patients, with an overall number of 1482 valid TDM measurements, met the inclusion criteria. Patient characteristics and genotype frequencies of *CYP2C19*, *CYP2C:TG*, and *CYP2B6* are presented in Table 1. All investigated variants (single nucleotide polymorphisms, SNPs) were in Hardy Weinberg equilibrium and the frequencies were in concordance with frequencies observed in Europeans in general.²²

Effect of *CYP2C19* genotypes on the sertraline concentration

The impact of the *CYP2C:TG* haplotype and *CYP2C19* variants on sertraline serum concentration was investigated

TABLE 1 Patient demographics for each diplotype

Phenotype	Genotype	Patients, n (%)	Samples, n	Women, n (%)	Age, years, mean (SD)	Drug dosage, mg/day, mean (SD)	Time between last drug intake and blood sampling (h), mean (SD)
UM	CYP2C19*17/CYP2C19*17	44 (5.2)	89	31 (70.5)	41.3 (17.2)	115.5 (60.2)	20.3 (5.75)
	CYP2C: TG/CYP2C: TG	26 (3.1)	34	14 (53.9)	45.2 (20.0)	97.1 (35.8)	21.1 (4.54)
	CYP2C: TG/CYP2C19*17	65 (7.7)	108	43 (66.2)	51.1 (19.7)	120.7 (71.0)	21.0 (5.64)
	CYP2C19*1/CYP2C19*17	150 (17.9%)	286	103 (68.7)	45.8 (18.1)	145.0 (126.0)	19.9 (5.31)
RM	CYP2C19*1/CYP2C: TG	142 (16.9)	222	95 (66.9)	44.2 (17.9)	111.6 (53.3)	20.4 (5.21)
	CYP2C19*1/CYP2C19*1 (Ref.)	160 (19.1)	288	99 (61.9)	44.2 (18.6)	106.3 (51.4)	20.2 (5.68)
IM	CYP2C19*17/CYP2C19	55 (6.6)	93	33 (60.0)	46.4 (18.4)	113.6 (57.5)	20.0 (5.74)
	CYP2C: TG/CYP2C19	61 (7.3)	120	41 (67.2)	43.9 (18.7)	98.0 (48.4)	18.7 (5.69)
	CYP2C19*1/CYP2C19	108 (12.9)	167	69 (63.9)	44.9 (18.5)	100.8 (53.0)	21.4 (5.10)
	CYP2C19 Null/CYP2C19	29 (3.5)	75	21 (72.4)	34.0 (14.3)	110.5 (47.3)	18.9 (5.46)
PM	Total	840	1482	549 (65.4)	44.6 (18.4)	115.3 (75.3)	20.2 (5.48)
	CYP2B6*1/*4	33 (4.2)	62	21 (63.6)	42.1 (16.0)	117.3 (61.2)	20.7 (6.04)
	CYP2B6*4/*4	4 (0.5)	8	3 (75.0)	63.8 (21.8)	150.0 (46.3)	18.3 (5.80)
	CYP2B6*1/*1 (Ref.)	454 (57.3)	848	297 (65.4)	43.8 (17.9)	121.7 (87.2)	20.1 (5.41)
IM	CYP2B6*1/*6	251 (31.7)	383	162 (64.5)	42.7 (18.3)	106.1 (50.9)	20.5 (5.54)
	CYP2B6*1/*9	3 (0.4)	7	3 (100)	45.3 (11.2)	132.1 (23.8)	24.2 (1.36)
	CYP2B6*4/*6	7 (0.9)	13	5 (71.4)	54.5 (22.8)	107.7 (78.7)	19.4 (5.90)
	CYP2B6*6/*6	39 (4.9)	46	24 (61.5)	45.7 (19.6)	87.0 (52.2) ^a	20.3 (5.47)
PM	CYP2B6*9/*9	1 (0.1)	2	1 (100)	63.5 (0.7)	100.0 (0)	23.4 (0.14)
	Total	792	1369	516 (65.2)	43.7 (18.1)	116.0 (76.5)	20.2 (5.48)

Note: Null = no function allele (i.e., the presence of CYP2C19*2, CYP2C19*3, or CYP2C19*4). Ref. = reference genotype.

Abbreviations: IM, intermediate metabolizer; NM, normal metabolizer; PM, poor metabolizer; RM, rapid metabolizer; UM, ultrarapid metabolizer.

^aIndicates significant difference ($p < 0.05$) compared with patients with the reference genotype (CYP2C19*1/*1 or CYP2B6*1/*1), patients not carrying any of the investigated variants.

using multivariate linear mixed-model analysis, adjusting for *CYP2B6* genotype, age, sex, and time between last dose and blood sampling. *CYP2D6* and *CYP2C9* genotypes had no significant impact on the dose-adjusted serum concentrations of sertraline ($p > 0.1$) and were therefore excluded from the statistical analysis.

Results of the mixed-model analysis estimating the quantitative effect of the *CYP2C:TG* haplotype and *CYP2C19* variants on sertraline concentration are presented in Table 2 and Figure 1. Compared with the reference group (*CYP2C19*1/*1*), a lower sertraline serum concentration was observed in *CYP2C19*17/*17* (21.6% decrease, $n = 44$, $p = 0.003$), *CYP2C:TG/CYP2C:TG* (21.2% decrease, $n = 26$, $p = 0.022$), *CYP2C19*17/CYP2C:TG* (20.0% decrease, $n = 65$, $p = 0.003$), and *CYP2C19*1/*17* (17.0% decrease, $n = 150$, $p < 0.001$) patients, while no significant impact of *CYP2C19*1/CYP2C:TG* genotype was detected in this patient population ($n = 142$, $p > 0.1$).

Compared to the reference group (*CYP2C19*1/*1*), patients homozygous for the non-functional *CYP2C19* alleles (**2*, **3*, or **4*) had a 2.3-fold ($n = 29$, $p < 0.001$) increase in serum concentrations of sertraline (Table 2). Compared to the reference group, patients heterozygous for the non-functional alleles of *CYP2C19* in combination with either *CYP2C:TG* or *CYP2C19*17* had 1.21-fold ($n = 61$, $p = 0.01$) and 1.37-fold ($n = 55$, $p < 0.001$) increased sertraline concentration, respectively (Table 2).

Effect of *CYP2B6* genotypes on the sertraline concentration

The impact of the *CYP2B6* variants on sertraline serum concentration was investigated using multivariate linear mixed-model analysis, adjusting for *CYP2C:TG*, *CYP2C19* genotype, age, sex, and time between last dose and blood sampling. Compared with the reference group

TABLE 2 Impact of *CYP2C19* and *CYP2B6* genotype on sertraline concentration estimated in the fixed effect mixed-model analysis

Phenotype	Genotype	Patients, n (%)	Samples, n	Sertraline		
				Predicted mean serum concentration, nM/100 mg day (95% CI)	Fold difference (95% CI)	p -Value
CYP2C19						
NM	<i>CYP2C19*1/*1</i> (Ref.)	160 (19.1)	288	71.6 (62.5–82.13)	-	-
UM	<i>CYP2C19*17/CYP2C19*17</i>	44 (5.2)	89	56.2 (47.8–66.0)	0.78 (0.67–0.92)	0.003
	<i>CYP2C:TG/CYP2C:TG</i>	26 (3.1)	34	56.5 (46.1–69.2)	0.79 (0.64–0.97)	0.022
	<i>CYP2C:TG/CYP2C19*17</i>	65 (7.7)	108	57.3 (49.4–66.4)	0.80 (0.69–0.93)	0.003
RM	<i>CYP2C19*1/CYP2C19*17</i>	150 (17.9)	286	59.5 (53.3–66.4)	0.83 (0.74–0.93)	<0.001
	<i>CYP2C19*1/CYP2C:TG</i>	142 (16.9)	222	72.2 (64.5–80.8)	1.01 (0.90–1.13)	0.893
IM	<i>CYP2C19*17/CYP2C19 Null</i>	55 (6.6)	93	74.1 (63.8–86.1)	1.03 (0.89–1.20)	0.661
	<i>CYP2C:TG/CYP2C19 Null</i>	61 (7.3)	120	86.6 (75.0–100.0)	1.21 (1.05–1.40)	0.010
	<i>CYP2C19*1/CYP2C19 Null</i>	108 (12.9)	167	97.8 (86.8–110.3)	1.37 (1.21–1.54)	<0.001
PM	<i>CYP2C19Null/CYP2C19 Null</i>	29 (3.5)	75	163.3 (134.6–198.1)	2.28 (1.88–2.77)	<0.001
CYP2B6						
NM	<i>CYP2B6*1/*1</i> (Ref.)	454 (57.3)	848	71.6 (62.5–82.1)	-	-
UM	<i>CYP2B6 (*1/*4, *4/*4)</i>	37 (4.7)	70	59.2 (50.2–69.7)	0.83 (0.70–0.97)	0.022
IM	<i>CYP2B6 (*1/*6, *1/*9, *4/*6)</i>	261 (33.0)	403	82.3 (76.5–88.5)	1.15 (1.07–1.24)	<0.001
PM	<i>CYP2B6 (*6/*6, *9/*9)</i>	40 (5.1)	48	89.4 (75.9–105.3)	1.25 (1.06–1.47)	0.008

Note: A 1.9-fold difference ($p = 0.019$) and a 1.18-fold difference ($p < 0.001$) in sertraline concentration were observed for women and patients aged >65 years, respectively. Null = no function allele (i.e. the presence of *CYP2C19*2*, *CYP2C19*3*, or *CYP2C19*4*).

Ref. = reference genotype.

Genotype is used as fixed effects in the mixed model. The sertraline concentration of patients not carrying any of the analyzed variants of *CYP2C19* or *CYP2B6* were set as the reference group. Fold difference represents the estimated relative impact of the occurrence of the genotype on sertraline concentration, as compared with the reference group. Serum concentrations are predicted from the mixed-model analysis, with age <65 years and sampling time 20 h as model determined covariate values.

Abbreviations: CI, confidence interval; IM, intermediate metabolizer; NM, normal metabolizer; PM, poor metabolizer; RM, rapid metabolizer; UM, ultrarapid metabolizer.

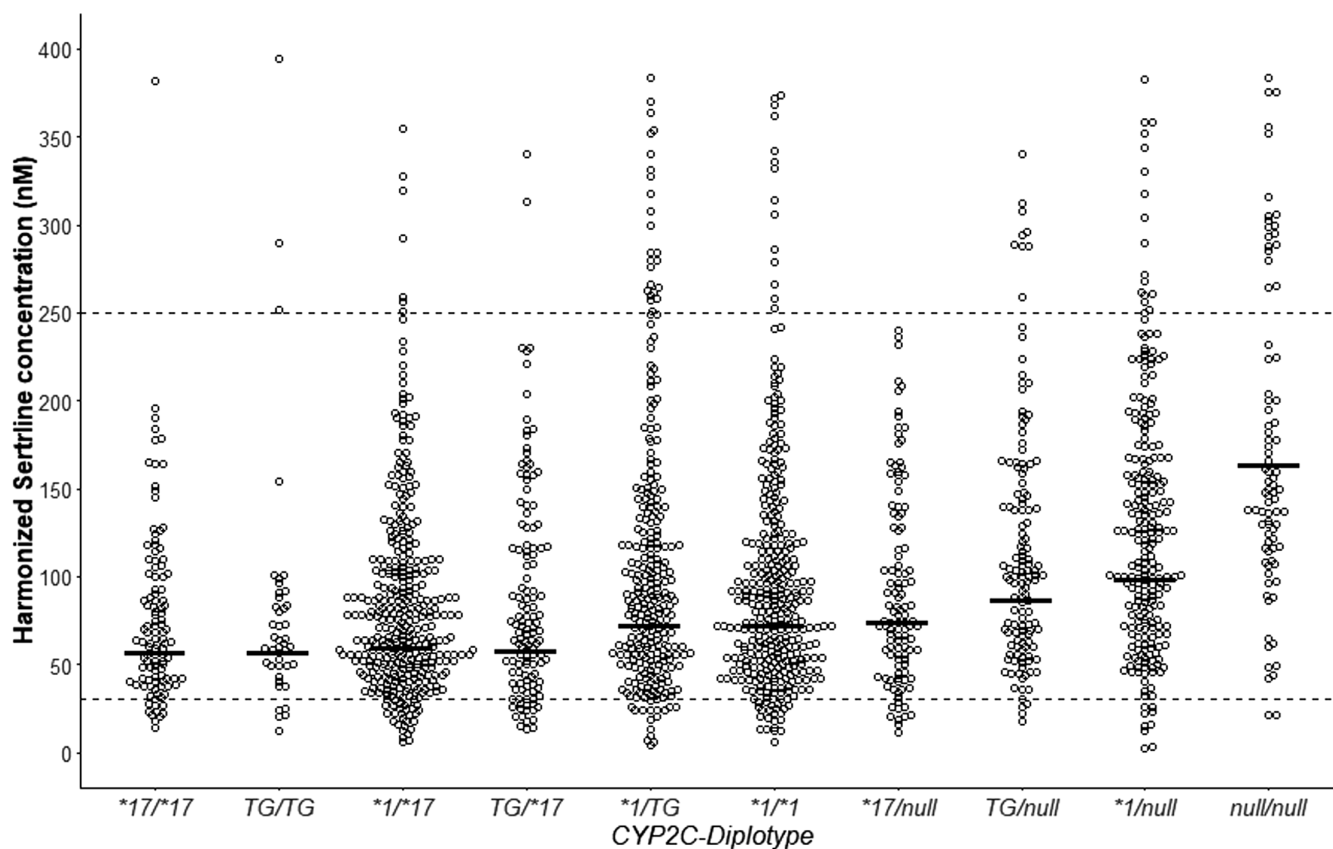


FIGURE 1 Sertraline serum concentration measurements in patient subgroups carrying different *CYP2C19* genotypes in combination with *CYP2C:TG*, where the crossbar indicates the predicted mean serum concentration for each diplotype. Sertraline serum concentrations were normalized to the dosage of 100 mg/day. The predicted mean serum concentration is based on mixed-model analyses of the different *CYP2C19* genotypes and is presented as crossbar for each *CYP2C19* diplotype. Patients with a *CYP2C19**1/*1 genotype were used as reference in the mixed-model analysis. Null = no function allele (i.e., presence of *CYP2C19**2, *CYP2C19**3, or *CYP2C19**4). The dotted line indicates the therapeutic reference range (30–250 nM).

(*CYP2B6**1/*1, $n = 454$), patients carrying the *CYP2B6**4 allele had a 17.4% ($n = 37$, $p = 0.022$) reduced serum concentration of sertraline (Table 2). On the contrary, compared to the reference group, patients homozygous or heterozygous for the *CYP2B6**6 or *9 allele had a 25% ($n = 40$, $p = 0.008$) and 15% ($n = 261$, $p < 0.001$) increased serum concentration of sertraline, respectively (Table 2).

In addition, a significant impact of sex and age on sertraline serum concentration was found in the patient population, where a 11% ($p < 0.001$) and 18% ($p < 0.001$) increased serum concentration of sertraline was found for women and patients >65 years ($n = 176$), respectively.

Effect of genotype-predicted phenotype subgroups on sertraline exposure

Patients were further divided into genotype-predicted phenotype subgroups to investigate the combined effect

of *CYP2C19* and *CYP2B6* phenotypes on the sertraline serum concentration which was investigated using a multivariate linear mixed model, adjusting for age, sex, and time between last dose and blood sampling. Patients with a normal metabolizer phenotype of both *CYP2C19* and *CYP2B6* were used as reference. Results of the mixed-model analysis based on genotype-predicted phenotype subgroups is presented in Table S1.

Predicted mean serum concentration of sertraline for the different combinations of *CYP2C19* and *CYP2B6* phenotypes were calculated using the estimates from the phenotype model, and the results are presented in Table 3 and Figure 2. Most notably, the predicted sertraline serum concentration in patients with a combined ultrarapid metabolizer (*CYP2C19* UM+ *CYP2B6* UM) phenotype was reduced by 35.4%, compared with the reference group (*CYP2B6* NM and *CYP2C19* NM). Patients with a combined poor metabolizer (*CYP2C19* PM+*CYP2B6* PM) phenotype had a 2.89-fold increased predicted mean serum concentration, compared to the reference group.

TABLE 3 Predicted mean serum concentration of sertraline in combined genotype-predicted phenotype groups of CYP2B6 and CYP2C19

Phenotype				Sertraline	
CYP2B6	CYP2C19	Patients, <i>n</i>	Samples, <i>n</i>	Predicted mean serum concentration, nM/100 mg day (95% CI)	Fold difference (95% CI)
UM	UM	10	22	46.31 (35.0–61.3)	0.65 (0.49–0.86)
UM	RM	6	7	53.17 (40.9–69.1)	0.74 (0.57–0.96)
UM	NM	9	19	58.52 (49.6–69.1)	0.82 (0.69–0.96)
UM	IM	9	17	72.19 (55.3–94.2)	1.01 (0.77–1.32)
UM	PM	1	1	133.28 (92.9–191.2)	1.86 (1.30–2.67)
NM	UM	67	123	56.71 (50.5–63.6)	0.79 (0.71–0.89)
NM	RM	156	281	65.11 (59.1–71.7)	0.91 (0.83–1.00)
NM	NM	90	155	71.66 (62.4–82.3)	–
NM	IM	119	224	88.4 (79.9–97.8)	1.23 (1.12–1.36)
NM	PM	14	52	163.21 (121.7–198.5)	2.28 (1.87–2.77)
IM	UM	40	60	65.64 (54.4–79.2)	0.92 (0.76–1.11)
IM	RM	93	144	75.37 (63.6–89.3)	1.05 (0.89–1.25)
IM	NM	43	72	82.95 (77.1–89.3)	1.16 (1.08–1.25)
IM	IM	73	110	102.33 (86.0–121.8)	1.43 (1.20–1.70)
IM	PM	10	15	188.92 (144.4–247.2)	2.64 (2.01–3.45)
PM	UM	6	7	71.99 (54.4–95.3)	1.01 (0.76–1.33)
PM	RM	14	18	82.65 (63.6–107.4)	1.15 (0.89–1.50)
PM	NM	5	6	90.96 (77.1–107.3)	1.27 (1.08–1.50)
PM	IM	10	10	112.22 (86.1–146.3)	1.57 (1.20–2.04)
PM	PM	2	2	207.18 (144.5–297.1)	2.89 (2.02–4.15)

Note: Patients with a CYP2B6 NM and CYP2C19 NM phenotype were set as the reference group. Fold difference represents the estimated relative impact of the occurrence of the combination of phenotype on sertraline serum concentration, as compared with the reference group.

Abbreviations: CI, confidence interval; IM, intermediate metabolizer; NM, normal metabolizer; PM, poor metabolizer; RM, rapid metabolizer; UM, ultrarapid metabolizer.

Formation of *N*-desmethylsertraline in relation to *CYP2C19* and *CYP2B6* genotypes

In addition to sertraline exposure, the formation of the inactive metabolite *N*-desmethylsertraline, in relation to *CYP2C19* and *CYP2B6* genotypes, was also analyzed (Table S2). The metabolite was previously reported to be formed by *CYP2C19* and *CYP2B6* to a limited extent,^{3,23} which was supported by the only 1.2-fold higher *N*-desmethylsertraline-to-sertraline metabolic ratio (MR) in CYP2C19 PMs versus NMs (Table S3). For CYP2B6, IMs and PMs had similar MR as NMs (Table S3). The lack of effect of *CYP2C19* and *CYP2B6* genotypes on the rate of formation of *N*-desmethylsertraline reflects the fact that the levels of this metabolite were directly proportional to sertraline exposure, that is, enzymatic access of the substrate sertraline in relation to the respective genotypes (Table S2), and indicates that enzymes other than CYP2C19 and CYP2B6 are responsible for the *N*-desmethylsertraline formation, presumably CYP3A4.⁷

DISCUSSION

In this study we have investigated the impact of *CYP2C19* and *CYP2B6* variants on sertraline serum levels, with the emphasis on the novel *CYP2C:TG* haplotype (rs2860840T and rs11188059G), in a large population of psychiatric patients. The results demonstrate that patients carrying the *CYP2C:TG* haplotype, which was previously suggested to encode increased CYP2C19 metabolic capacity,¹⁸ had a lower serum concentration of sertraline, and that the decrease was similar as in the patients carrying the validated increased expression allele *CYP2C19*17*. These findings are in line with those recently reported based on another CYP2C19 substrate, escitalopram,¹⁹ and hence these data together support the hypothesis that the *CYP2C:TG* haplotype is indeed associated with ultrarapid CYP2C19 metabolism. With a total population frequency of 10% for *CYP2C:TG/CYP2C:TG* and *CYP2C:TG/CYP2C19*17*, our discovery

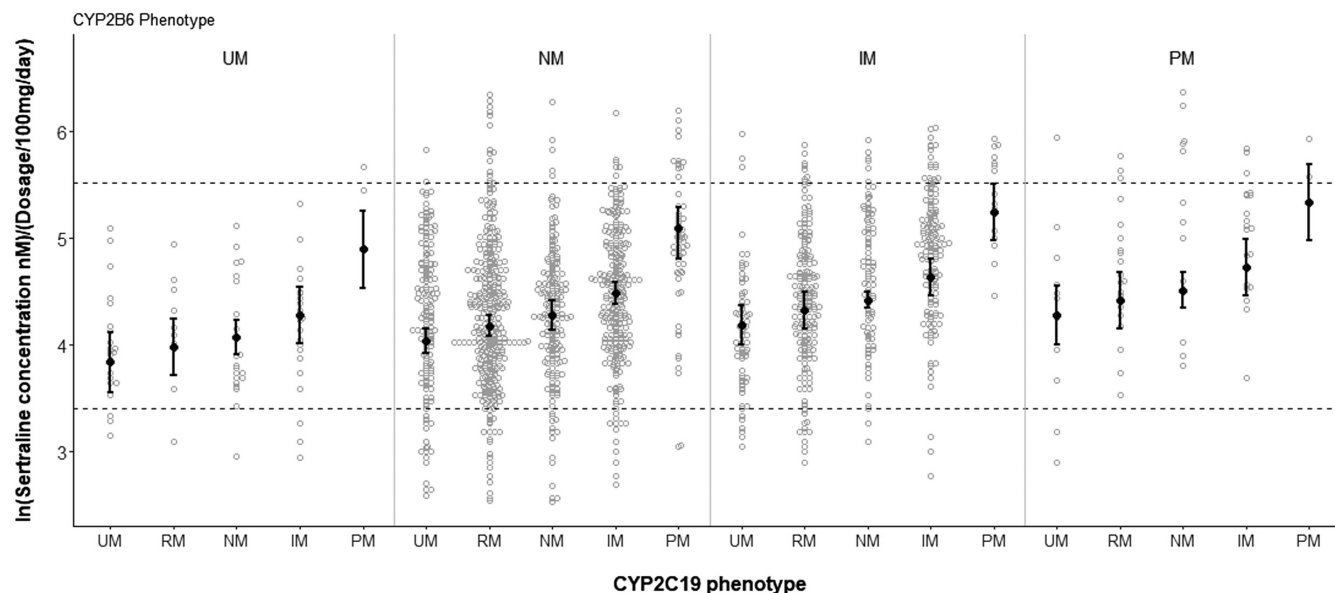


FIGURE 2 Sertraline serum concentration measurements in different phenotype subgroups of CYP2C19 and CYP2B6. The sertraline serum concentrations were normalized to the dosage of 100 mg/day and analyzed in the natural logarithmic scale. Predicted serum concentration is based on mixed-model analyses of the different CYP2C19 and CYP2B6 phenotype subgroups and is presented as error bars for each phenotype combination. The dotted line indicates the therapeutic reference range (30–250 nM).

of *CYP2C:TG* may be of substantial clinical importance for the treatment of sertraline and potentially additional CYP2C19 substrates.

These results together imply that patients homozygous for either *CYP2C19*17* or *CYP2C:TG* or a combination of both generally would require higher sertraline dosing than *CYP2C19*1/*1* carriers to reach therapeutic concentrations. Furthermore, this study indicated that *CYP2B6* genotype also significantly contributed to the individual variability in exposure and metabolism of sertraline, albeit at a lower extent as compared to variants encoding non-functional *CYP2C19* allelic variants. Regarding clinically personalized sertraline dosing, preemptive genotyping of *CYP2C19*, the *CYP2C:TG* haplotype, and *CYP2B6* in sertraline-treated patients may be more informative. Most notably, a group which may be of particular risk of sertraline underexposure and treatment failure are the patients with genotypes predicting UM phenotypes of both CYP2C19 and CYP2B6; 1.2% of the European population fall into this category associated with extremely fast sertraline clearance. Based on the results of the present study, such patients would require higher doses than recommended to ascertain optimal sertraline exposure. However, further studies are necessary to provide evidence for the clinical relevance of this pharmacokinetic/pharmacogenetic interpretation.

In a previous study, we observed a minor, but statistically significant lower serum concentration of sertraline of 8% in patients carrying the *CYP2C19*17* allele compared with the reference group.⁵ However, the reference group

(*CYP2C19*1/*1*) in the previous study was in fact a mixture of NMs and the, at that time, unknown RMs (*CYP2C19*1/CYP2C:TG*) and UMs (*CYP2C:TG/CYP2C:TG*). Therefore, in the present study, where the *CYP2C:TG* haplotype was considered and when the reference group contained only prototypical *CYP2C19*1/*1* NMs, the mentioned difference in sertraline exposure between CYP2C19 NM and UM increased to 22%. Accordingly, the *CYP2C:TG* haplotype identified among *CYP2C19*1/*1* patients¹⁹ may explain the previously observed underexposure of sertraline in CYP2C19 NMs, in a similar pattern as for the underexposure of escitalopram described for a substantial number of *CYP2C19*1/*1* carriers reported by Jukic et al.¹⁰

The molecular mechanism underlying the increased metabolism of CYP2C19 substrates caused by *CYP2C:TG* is unclear. Since CYP2C19 also metabolizes a multitude of other types of drugs such as proton pump inhibitors, diazepam, clopidogrel, voriconazole, diazepam, proguanil, and many others it appears of importance to consider the *CYP2C:TG* haplotype when conducting genotype phenotype linkage analyses for all these drugs.

In addition, the significant effect of *CYP2B6* genotype on the serum concentration of sertraline shown in this study may have clinical relevance. Patients carrying the *CYP2B6*4* allele exhibited a 17% reduced sertraline concentration, which is similar to the effect observed in CYP2C19 UM patients. There is a lack of in vivo data on the effect of *CYP2B6*4* on sertraline metabolism, but studies investigating the effect of this variant on other CYP2B6 substrates have already indicated that it causes

increased enzyme activity.¹⁴ Regarding the effects of *CYP2B6**6 or *9 alleles on sertraline metabolism, patients who were homo- or heterozygous carriers exhibited a 1.15–1.30-fold increased serum concentration of sertraline, respectively. Regarding increased risk of overexposure and adverse effects, these differences alone are unlikely to be of major clinical relevance; however, combined with the presence of *CYP2C19* PM genotypes, the impact of *CYP2B6**6 or *9 may be relevant for sertraline dosing decisions.⁵ For example, patients with a combined poor metabolizer (*CYP2C19* PM + *CYP2B6* PM) phenotype had a 2.89-fold increased serum concentration, compared to the reference group. Overall, the present study confirms that *CYP2B6* phenotype is an important determinant for the serum concentration and dose requirement of sertraline and that it should not be neglected as it currently is. Thus, individualized sertraline dosing based on preemptive genotyping of *CYP2C19/CYP2B6* and/or TDM can be used to minimize the over- and under-exposure of sertraline.

This study has some inherent limitations associated with the retrospective use of the TDM data. One is that information about comedication relies on the information provided by the physicians on the requisition form. The potential comedication with *CYP2C19* inhibitors could be missed, although concurrent use of enzyme-inducing anti-epileptics, which were excluded from the study, is often specified on the requisition form. This represents a potential non-pharmacogenetic source of variability in this patient population. Only common *CYP2B6* allelic variants were investigated in this study; further studies investigating the effect of *CYP2B6* variants on sertraline metabolism might also include other rare *CYP2B6* variants in order to increase the resolution of the influence of *CYP2B6* genotypes. This study did not include *CYP3A4*, whereas consideration of *CYP3A4* may be relevant to sertraline dosing. Further, other factors possibly affecting the serum concentration of sertraline (e.g., body weight, renal function, liver function, inflammatory state, somatic diseases, and/or partial nonadherence) could not be controlled for. However, the potential interference of these confounding factors is likely outweighed by the large number of included patients.

CONCLUSIONS

Patients carrying the *CYP2C:TG*-haplotype demonstrate similar reduced exposure of sertraline as the increased expression allele *CYP2C19**17, that is, about 20% in homozygous carriers, respectively. These patients will generally require a 25% higher dose to obtain the full

potential effect of sertraline. Furthermore, the study also reveals a significant impact of *CYP2B6* genotype on sertraline exposure. Consequently, patients with a combined homozygous presence of *CYP2C19**17 and *CYP2C:TG* haplotype as well as *CYP2B6**4 are expected to require a 50% increased dose to reach target concentration levels. Based on the relative differences in serum concentrations compared to NMs, dose reductions of 65% and 30% should be considered in patients with a combined PM/PM and IM/IM phenotype, respectively, to reduce the risk of sertraline overexposure in these patients. The significant impact of combined genotype-predicted phenotypes of *CYP2B6* and *CYP2C19*, including the novel *CYP2C*-haplotype, on sertraline concentration suggests that genotyping of both the *CYP2C:TG* and *CYP2B6* (*4, *6, and *9) should be included in genotyping panels for sertraline metabolism, in addition to *CYP2C19* genotyping. Further studies are necessary to elucidate the clinical implications of the novel *CYP2C*-haplotype for the treatment of >20 drugs having pharmacogenomic labels for *CYP2C19* variants, including substrates like omeprazole, voriconazole, and clopidogrel.

AUTHOR CONTRIBUTIONS

L.S.B., E.M., M.K.K., M.I.S., and M.M.J. wrote the manuscript. L.S.B, M.K.K., and E.M. designed the research. L.S.B. performed the research. L.S.B., E.M., M.I.S., M.M.J., and M.K.K. analyzed the data.

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CONFLICT OF INTEREST

The authors declared no competing interests for this work.

ORCID

Line Skute Bråten  <https://orcid.org/0000-0001-6887-1986>

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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