The Relevance of Integrating CYP2C19 Phenoconversion Effects into Clinical Pharmacogenetics

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ABSTRACT

Introduction CYP2D6 and CYP2C19 functional status as defined by genotype is modulated by phenoconversion (PC) due to pharmacokinetic interactions. As of today, there is no data on the effect size of PC for CYP2C19 functional status. The primary aim of this study was to investigate the impact of PC on CYP2C19 functional status.

Methods Two patient cohorts (total n = 316; 44.2 ± 15.4 years) were investigated for the functional enzyme status of CYP2C19 applying two different correction methods ($PC_{Bousman}$, $PC_{Hahn\&Roll}$) as well as serum concentration and metabolite-to-parent ratio of venlafaxine, amitriptyline, mirtazapine, sertraline, escitalopram, risperidone, and quetiapine.

Results There was a decrease in the number of normal metabolizers of CYP2C19 and an increase in the number of poor metabolizers. When controlled for age, sex, and, in the case of amitriptyline, venlafaxine, and risperidone, CYP2D6 functional enzyme status, an association was observed between the CYP2C19 phenotype/functional enzyme status and serum concentration of amitriptyline, sertraline, and escitalopram.

Discussion PC of CYP2C19 changes phenotypes but does not improve correlations with serum concentrations. However, only a limited number of patients received perturbators of CYP2C19. Studies with large numbers of patients are still lacking, and thus, it cannot be decided if there are minor differences and which method of correction to use. For the time being, PC is relevant in individual patients treated with CYP2C19-affecting drugs, for example, esomeprazole. To ensure adequate serum concentrations in these patients, this study suggests the use of therapeutic drug monitoring.

Introduction

For pharmacogenetic (PGx) considerations in psychopharmacological treatment, clinical recommendations are available for patients treated with tricyclic antidepressants and selective serotonin reuptake inhibitors, which specify how to adjust dosages according to the CYP2D6 and CYP2C19 phenotypes of the patient [1–4]. Currently, the genotype-inferred phenotypes are primarily considered [2, 4]. Concomitant drugs that inhibit cytochrome P450 (CYP) enzyme activity or induce their expression can cause phenoconversion (PC) effects. PC, therefore, leads to a discordance between the genotype-derived phenotype and the clinically observed phenotype (functional enzyme status) [5–10]. In our case, for example, bupropion, or fluoxetine (CYP2D6), and fluvoxamine, or fluoxetine (CYP2C19) [6, 7] are potential pertubators of relevant CYP enzymes. Experimental methods to measure PC in patients (for example, using the "Geneva Micrococktail" [11]) are not suitable for clinical routine; therefore, a method that does not interfere with the complex therapy of vulnerable psychiatric patients would be desirable. To address this, a calculator tool for CYP2D6 was established to integrate standardized assessments of PC in clinical practice [5, 7]. The activity score of CYP2D6 is multiplied by a factor corresponding to the inhibitory properties of the comedication (strong/moderate/weak). The adjusted activity score is then assigned to the adjusted phenotype [5]. As patients are routinely treated with multiple drugs in clinical practice, PC is common among psychiatric inpatients [12]. Considering the CYP2D6 functional enzyme status, the poor (PM) and intermediate status (IM) are much more common, and the normal metabolizer (NM) status is less common compared to the genotype-inferred phenotype [12–14]. For example, a patient genotyped as CYP2D6 NM treated with bupropion will phenoconvert to a CYP2D6 PM. Not considering PC in the interpretation of PGx results can lead to an inappropriate drug selection or false dosing recommendation, which in turn increases the risk for adverse drug reactions or nonresponse. Consequently, the phenoconversion effects of CYP2D6 are relevant [12–14]; however, integration in clinical routine is currently rare [5].

As of today, data on the relevance of PC for CYP2C19 are missing. One study described a decrease in CYP2C19 NM and an increase in IM when considering PC; however, the authors did not report statistical significance [13]. Unlike CYP2D6, different methods are available for CYP2C19 to correct for PC effects, taking into account the presence of an inducer or a moderate or strong inhibitor [7, 15, 16]. According to Bousman et al. [7], in the presence of a moderate CYP2C19 inhibitor, the phenotype is converted to the next lower activity, whereas a concomitant strong inhibitor leads to a conversion into a PM functional enzyme status regardless of the genotype-derived status. If an inducer is present, the phenotype is converted to the next higher activity phenotype. On the other hand, according to Hahn and Roll [17], in the presence of a moderate or strong inhibitor, NM and IM are phenoconverted to PM, whereas rapid (RM) and ultrarapid metabolizers (UM) are both converted to IM, respectively. In the presence of a moderate or strong inducer, NM and RM are phenoconverted to UM whereas IM is converted to NM. Thus, the latter method is stricter in the presence of a moderate CYP2C19 inhibitor. However, there is currently no consensus on any approach to adjust CYP2C19 phenotypes [5, 7, 16, 18]. Also, physiologically based pharmacokinetics modeling is an approach to predict phenoconversion effects [19]. A model predicting the phenoconversion of CYP2C19 by esomeprazole is available [19]; however, besides that, available models mainly focus on specific drug-drug interactions.

Aside from CYP2D6, CYP2C19 is an important enzyme in the metabolism of psychotropic drugs [20], and its phenotype affects serum concentrations of many drugs [21]. Mainly selective serotonin reuptake inhibitors and tricyclic antidepressants serum concentrations are affected by the CYP2C19 phenotypes [2, 4]; in addition, in a previous study, CYP2C19 phenotypes also affected venlafaxine serum concentration [22]. So far, studies reporting the pharmacokinetics of the drugs with respect to the CYP2C19 functional enzyme status in a clinical setting are missing.

To address these prevailing issues and therefore to improve the interpretation of PGx result on *CYP2C19*, the primary goal was to investigate how considering PC alters the CYP2C19 phenotype status. Different methods of including phenoconversion effects were applied to compare the effect of the correction method. According to Mostafa et al. [13, 15], PC should be calculated rather than measured to relieve psychiatric patients, but also to obtain results applicable to routine clinical practice. Second, as an exploratory goal, this study investigates how the CYP2C19 functional enzyme status affects serum concentrations and metabolite-to-parent ratios of psychotropic drugs.

Methods

Patients

Wuerzburg Sample

In the Wuerzburg sample, 212 inpatients at the Department of Psychiatry, Psychosomatics, and Psychotherapy of the University Hospital of Wuerzburg, with available genotype data, as well as therapeutic drug monitoring (TDM) results, were included in the analyses. Only adult patients (≥ 18 years of age) were included. Genotyping of *CYP2D6* and *CYP2C19*, as well as TDM, were performed according to the physician's choice as part of the clinical routine. TDM was performed according to the guidelines of the TDM expert group of the Arbeitsgemeinschaft für Neuropsychopharmakologie und Pharmakopsychiatrie (AGNP) [20]. Genotyping for *CYP2D6* and *CYP2C19* was performed according to recommendations of the German Genetic Diagnostics Commission [23, 24] and according to the procedures of the German Genetic Diagnostics Act with written informed consent. Genotypes and serum concentrations were determined between January 2020 and December 2021. To avoid bias in case of multiple serum concentration determinations for one drug in the same patient, only the latest determination per analyte was included in the analyses. The retrospective analysis of clinical routine data was approved by the Wuerzburg ethics committee (20220120 02) and was performed in accordance with the principles of the declaration of Helsinki.

Frankfurt Sample

Adult inpatients (≥ 18 years of age) admitted to the Department of Psychiatry, Psychosomatic Medicine and Psychotherapy of the University Hospital Frankfurt due to a depressive episode (single

major depressive episode, recurrent depression) were genotyped for *CYP2D6* and *CYP2C19* as part of the FACT-PGx study. TDM was performed as part of the clinical routine according to the physician's choice according to the guidelines of the TDM expert group of the AGNP [20]. Data of 104 patients who took part in the FACT-PGx study with available TDM data were included in the analyses. Genotypes and serum concentrations were determined between July 2021 and March 2022. To avoid bias in case of multiple serum concentration determinations for one drug in the same patient, only the latest determination per analyte was included in the analyses. The study was approved by the local ethics committee of the University of Frankfurt (2021–138) and carried out in accordance with the ethical principles of the Helsinki Declaration version 2013. Written informed consent was obtained from each participant.

Genotyping and therapeutic drug monitoring

Genotyping and serum concentration determinations in both cohorts were performed at the Department of Psychiatry, Psychosomatics, and Psychotherapy of the University Hospital of Wuerzburg. Details about the methods are available in **Supplement 1**.

Haplotypes were defined for all analyzed single nucleotide polymorphisms according to gene-specific haplotype tables from the PharmVar homepage (https://www.pharmvar.org/genes; **Supplement 1**). Phenotypes were determined according to the Clinical Pharmacogenetics Implementation Consortium (CPIC) specifications [25].

Dose-corrected serum concentrations (serum concentration/dose, CD) of either the active moiety of the drug (serum concentration parent drug + active metabolite; CD_{AM}) or the parent drug alone, depending on the relevance for treatment response [20] and metabolite-to-parent ratios (MPR) were calculated [20].

Dimensional outliers (≥ 3 SD from the mean) from CD and MPR were set as missing data.

Phenoconversion effects

As there is no consensus on how to correct for the phenoconversion effects of CYP2C19 without using a "drug-cocktail" [11], two available methods were used and compared to each other. The phenoconversion effects were assessed according to Bousman et al. [7] and Hahn and Roll [17]. For details, see Introduction, and **Supplement 1**.

According to Bousman et al. [7], concomitant drugs with the propensity to cause phenoconversion due to inhibitory or inducing effects on CYP2C19 were derived from the Flockhart table (**Supplement 2**) [6]. For supplemental analysis, drugs with inhibitory and inducing effects on CYP2C19 were derived from the FDA table [26] (**Supplement 3**).

Statistical analyses

Statistical analyses were conducted in R v4.0.4 [27].

Differences in the CYP2C19 functional enzyme status obtained by different correction methods were investigated by performing McNemar tests with continuity correction. We performed Benjamini-Hochberg correction, as Bonferroni correction tends to be too conservative for genomic analysis due to the linkage equilibrium between individual genotypes [28]. A p-value < 0.05 was considered significant.

Differences in CD and MPR depending on the CYP2C19 functional enzyme status, were investigated by performing linear regression analyses, corrected for age and sex. In the amitriptyline, venlafaxine, and risperidone samples, the CYP2D6 functional enzyme status was also included in the regression analyses, as the serum concentrations of these drugs are also dependent on CYP2D6 functional enzyme status [12]. Chi-squared tests or Fisher's exact tests were performed to investigate the association between the phenotype and the serum concentration being below, above, or within the therapeutic reference range [20] for the respective drug. To obtain reliable statistic results, groups (below, above, or within the therapeutic reference range) with less than five patients were excluded from analyses. A p-value < 0.05 was considered significant.

Results

Patient Samples

The combined sample comprised 316 patients, which were 44.2 ± 15.4 (mean ± standard deviation (SD)) years old, and 54.1 % female. Among these, 144 patients were nonsmokers, 99 were smokers, and from 73 patients, no information on smoker status was available. Patients received between 0 and 18 additional drugs in combination (mean ± SD 4.1 ± 3.5). A more detailed demographic overview is given in ▶ **Table 1**. Eighteen patients were identified as CYP2C19 UM (genotype-inferred phenotype), 95 patients as RM, 129 as NM, 69 as IM, and 5 as PM.

The number of serum concentration determinations is listed in ▶ **Table 1**. Only patients who received venlafaxine (N = 117), amitriptyline (N = 100), mirtazapine (N = 85), sertraline (N = 64), escitalopram (N = 52), risperidone (N = 73), and quetiapine (N = 125) were included in the analyses to limit the type II error probability. Demographic data of these patients are given in **Supplement 4**. To increase statistical power, all analyses were performed in the combined sample.

Phenoconversion Effect

Results on phenoconversion effects are given per TDM request, as concomitant drugs with each TDM request affect the genotype-inferred phenotype.

At baseline, 40.9 % of the patients were classified as CYP2C19 NM (▶ **Table 2**); after accounting for PC, according to Bousman et al. ($PC_{Bousman}$) [7], the number significantly decreased, and 39.5 % were classified as NM_{PC} (p = 0.05) (▶ **Table 3**). According to Hahn and Roll ($PC_{Hahn\&Roll}$) [17], the number of NM changed not significantly (p = 0.08) (▶ **Table 3**); however, the number of PM significantly increased from 1.1 % to 2.7 % (p < 0.001). The number of IM, UM, and RM did not change significantly with either of the correction methods (▶ **Table 2**, **3**; ▶ **Figure 1**). Patients prone to PC are summarized in **Supplement 5**. As only five patients with CYP2C19 affecting concomitant medications according to the FDA phenoconversion list were included, the number of NM, IM, PM, RM, and UM did not change significantly after considering PC (**Supplement 3**).

▶ **Table 1** Demographic data of the patients included in the sample. Genotypic phenotypes were the phenotypes according to the PGx results.

		Combined Sample			
		N	Mean ± SD (range)		
INCLUDED PATIENTS		316			
AGE [YEARS]		316	44.2 ± 15.4 (18–84)		
MALE/FEMALE		145/171			
NONSMOKER/SMOKER		144/99			
	NONSMOKER M/F	57/87			
	SMOKER M/F	56/43			
MEDICATION WITH TDM					
ANTIDEPRESSANTS	**N**	**ANTIPSYCHOTICS**	**N**	**ANTIEPILEPTICS**	**N**
Venlafaxine	117	Quetiapine	125	Pregabaline	25
Amitriptyline	100	Risperidone	73	Pipamperone	20
Mirtazapine	85	Aripiprazole	32	Valproic Acid	15
Sertraline	64	Olanzapine	20	Lamotrigine	13
Escitalopram	52	Cariprazine	10	Oxcarbazepine	9
Bupropion	38	Clozapine	8	Gabapentine	3
Trazodon	32	Chlorprothixene	7	Carbamazepine	2
Duloxetine	28	Amisulpride	5	Topiramate	2
Clomipramine	27	Haloperidol	5	Levetiracetam	1
Milnacipran	19	Perazine	4		
Doxepine	16	Melperone	3		
Trimipramine	5	Benperidol	2		
Fluoxetine	4	Flupentixol	2		
Moclobemid	3	Fluphenazine	1		
Citalopram	2				
Maprotiline	1				
Opipramole	1				
COMEDICATION		4.1 ± 3.5 (0–18)			
GENOTYPIC PHENOTYPES					
CYP2C19		316			
	UM/RM/NM/IM/PM (%)	18/95/129/69/5 (5.7/30.1/40.8/21.8/1.6)			

N, number of patients; (%), percentage number; SD, standard deviation; M, male; F, female; UM, ultrarapid metabolizer; RM, rapid metabolizer; NM, normal metabolizer; IM, intermediate metabolizer; PGx, pharmacogenetic; PM, poor metabolizer.

▶ **Table 2** Number of CYPC19 phenotypes and CYP2C19 functional enzyme status assessed by different methods (Hahn&Roll [17], and Bousman et al. [5]).

	ALL PATIENTS (PER TDM)
	N (%)
CYP2C19 nonPC	633
UM/RM/NM/IM/PM	29/185/259/153/7 (4.6/29.2/40.9/24.2/1.1)
CYP2C19 $PC_{Hahn\&Roll}$	633
UM/RM/NM/IM/PM	32/182/251/150/17 (5.1/28.9/39.7/23.7/2.7)
CYP2C19 $PC_{Bousman}$	633
UM/RM/NM/IM/PM	30/185/250/160/8 (4.7/29.2/39.5/25.3/1.3)

N, number of patients; (%), percentage number; nonPC, non-phenoconversion; PC, phenoconversion; UM, ultrarapid metabolizer; RM, rapid metabolizer; NM, normal metabolizer; IM, intermediate metabolizer; PM, poor metabolizer.

Venlafaxine

CD_{AM} and MPR of venlafaxine were not associated with genotype-inferred CYP2C19 phenotypes, functional enzyme $status_{Bousman}$, and functional enzyme $status_{Hahn\&Roll}$ (**Supplement 6**).

Genotype-inferred CYP2C19 phenotypes, as well as the functional enzyme status, were not associated with serum concentrations below, above, or within the therapeutic reference range (**Supplement 6**).

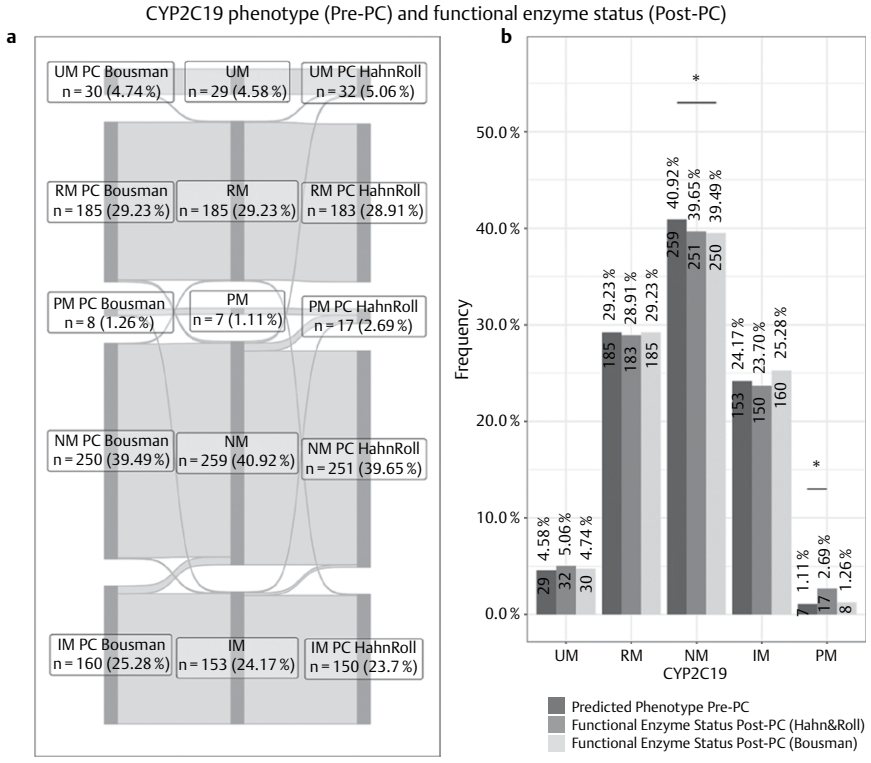
Amitriptyline

CD_{AM} of amitriptyline was associated with genotype-inferred CYP2C19 phenotypes, with RM and UM showing lower CD compared to NM ($ß_{std} = -0.52$, $p = 0.02$; $ß_{std} = -0.68$ $p = 0.04$) (**Supplement 6**). MPR was not associated with genotype-inferred CYP2C19 phenotypes, and these were not associated with serum concentrations below, above, or within the therapeutic reference range. Considering $PC_{Bousman}$ or $PC_{Hahn\&Roll}$ did not change the number of NM,

▶ **Table 3** CYP2C19 genotype-inferred phenotypes compared to functional enzyme status assessed by different methods (Hahn and Roll [17], and Bousman et al. [5]). McNemar test with continuity correction was used to describe significant differences in the number of phenotypes/functional enzyme status. Benjamini-Hochberg correction was performed, as Bonferroni correction tends to be too conservative for genomic analysis due to the linkage equilibrium between individual genotypes [30].

		N(nonPC)	$N(PC_{Hahn\&Roll})$	Adjusted p-value (unadjusted)	N(nonPC)	$N(PC_{Bousman})$	Adjusted p-value (unadjusted)
UM	UM nonUM	29 604	32 601	0.13 (0.08)	29 604	30 603	0.4 (0.32)
RM	RM nonRM	185 448	183 450	0.18 (0.16)	185 448	185 448	1.0 (1.0)
NM	NM nonNM	259 374	251 382	0.08 (0.03)	259 374	250 383	0.05 *(0.01)*
IM	IM nonIM	153 480	150 483	0.18 (0.18)	153 480	160 473	0.08 *(0.03)*
PM	PM nonPM	7 626	17 616	$7.85^{*}10^{-3}$ $(1.57^{*}10^{-3})$	7 626	8 625	0.4 (0.32)

N, number of patients; nonPC, non-phenoconversion; PC, phenoconversion; UM, ultrarapid metabolizer; RM, rapid metabolizer; NM, normal metabolizer; IM, intermediate metabolizer; PM, poor metabolizer.



▶ **Figure 1** (**a**) Sankey Plot showing the changes in CYP2C19 phenotypes when considering phenoconversion effects assessed by different methods (Hahn and Roll [17], and Bousman et al. [7]). (**b**) Frequencies of predicted CYP2C19 phenotype before (pre) and functional enzyme status after (post), including phenoconversion effects, are shown.

IM, PM, RM, and UM. Consequently, analyses on functional enzyme status were not performed (**Supplement 6**).

Mirtazapine

CD, as well as MPR of mirtazapine, were not associated with genotype-inferred CYP2C19 phenotypes, nor with functional enzyme status. Serum concentrations of mirtazapine within, above or below the respective therapeutic reference range were not associated with genotype-inferred CYP2C19 phenotypes, nor with functional enzyme status (**Supplement 6**).

Sertraline

CD of sertraline was associated with genotype-inferred CYP2C19 phenotypes with higher CD in PM compared to NM ($ß_{std} = 2.67$;

p = 0.005). A trend towards higher and lower CD in IM and UM, respectively, compared to NM, was observed ($ß_{std}$ = 0.74, p = 0.06; $ß_{std}$ = −0.94, p = 0.06). The number of NM, IM, PM, RM, and UM considering $PC_{Bousman}$ was concordant with the number considering $PC_{Hahn\&Roll}$. CD was associated with functional enzyme status with higher CD in PM compared to NM ($ß_{std}$ = 2.37, p < 0.001).

Metabolites were not measured; thus, analyses on MPR were not possible. Only one patient showed serum concentrations below the therapeutic reference range, and no patient showed concentrations above the reference range; therefore, further analyses could not be conducted (**Supplement 6**).

Escitalopram

CD of escitalopram was associated with genotype-inferred CYP2C19 phenotypes with lower CD in UM compared to NM ($ß_{std}$ = −1.96, p = 0.05). MPR of escitalopram was not associated with genotype-inferred CYP2C19 phenotypes. Genotype-inferred CYP2C19 phenotypes were associated with serum concentrations below or within the therapeutic reference range (p < 0.001). Post-hoc tests showed that frequencies of RM compared to IM were significantly different (p = 0.007). Considering $PC_{Bousman}$ or $PC_{Hahn\&Roll}$ did not change the number of NM, IM, and PM; RM, and UM, therefore, analyses on functional enzyme status were not performed (**Supplement 6**).

Risperidone

CD_{AM}, as well as MPR of risperidone, were not associated with genotype-inferred CYP2C19 phenotypes, nor with functional enzyme status. Serum concentrations of risperidone within, above, and below the respective therapeutic reference range were not associated with genotype-inferred CYP2C19 phenotypes, nor with functional enzyme status (**Supplement 6**).

Quetiapine

CD and MPR of quetiapine were not associated with genotype-inferred CYP2C19 phenotypes; also, serum concentrations of quetiapine within, above, and below the respective therapeutic reference range were not associated with genotype-inferred CYP2C19 phenotypes. Considering $PC_{Bousman}$ or $PC_{Hahn\&Roll}$ did not change the number of NM, IM, PM; RM, and UM, therefore, analyses on functional enzyme status were not performed (**Supplement 6**).

Discussion

In this naturalistic setting, we investigated how correcting for PC alters the CYP2C19 phenotype/functional enzyme status in a clinical routine setting. We applied different methods to correct for the phenoconversion effects, as there is no consensus on how to adjust CYP2C19 phenotypes yet [5, 7, 18]. Depending on the correction method, our findings reveal a significant decrease in CYP2C19 NM and a significant increase in PM. We explored the association between CYP2C19 functional enzyme status and the pharmacokinetics of antidepressant and antipsychotic drugs and found significant associations between drug exposure of amitriptyline, sertraline, and escitalopram and CYP2C19 phenotypes, as well as functional metabolizer status ($PC_{Bousman}$ and $PC_{Hahn\&Roll}$).

We applied two methods to calculate PC rather than measuring PC, e. g., by using the "Geneva Micrococktail" [11], to relieve the psychiatric patients, but still obtain results applicable to routine clinical practice.

CYP2C19 phenotype frequencies in our clinical routine sample are in concordance with the phenotype frequency for Europeans [29]. Less than one in two patients were CYP2C19 NM. When including $PC_{Bousman}$, in accordance with a previous study, the number of NM decreased; however, no statistical results were reported previously [13]. When applying $PC_{Hahn\&Roll}$, due to the stricter classification when taking a moderate CYP2C19 inhibitor, the number of PMs increased. Thus, the method of correction for PC significantly affected the frequencies of the functional enzyme status.

As including PC altered the frequencies of phenotypes/functional enzyme status of CYP2C19, PC is relevant for CYP2D6 [5, 12], and for CYP2C19; however, they may be less pronounced. PC rates in the present study seem much lower than in previous studies [13, 15]. Mostafa et al. included not only psychiatric patients [15]; in addition, esomeprazole was used more often in the previous study [13] compared to the present one. In clinical practice in Würzburg and Frankfurt, pantoprazole is preferred over (es)omeprazole due to the preferable drug interaction profile.

Compared to CYP2D6, CYP2C19-affecting drugs were less often used; only 17 patients (5.4 %) were prone to CYP2C19 PC; in contrast, 24.1 % of the patients were prone to CYP2D6 PC [12]. Thus, due to the limited use of CYP2C19-affecting drugs, PCs are less common; nevertheless, PCs are relevant for an individual treated with CYP2C19-inhibiting/inducing drugs, especially esomeprazole [6, 30]. Therefore, we suggest considering PC not only for CYP2D6, but also for CYP2C19 as part of individualized treatment in psychiatry.

Considering the FDA phenoconversion table, the number of NM, IM, PM, RM, and UM did not change significantly after taking into account PC. However, in the FDA phenoconversion table, esomeprazole is not considered a CYP2C19 inhibitor. This is in contrast to the product information of the European Medicines Agency (EMA) that esomeprazole is a CYP2C19 inhibitor, and when starting or ending treatment with esomeprazole, the potential for interactions with drugs metabolized through CYP2C19 should be considered [30]. Moreover, also clinical data showed that esomeprazole inhibits CYP2C19 clinically relevant [31, 32].

For CYP2D6, there is consensus among experts that if the patient is taking a strong or moderate inhibitor, the activity score of CYP2D6 should be multiplied by 0 or 0.5, respectively. Administration of a weak inhibitor does not require adjustment, as the area under the curve is only minimally affected by weak inhibitors [5, 33]. This concurs with the definition of the relevance of drug interactions in general, which are only considered relevant with moderate and strong inhibitors. In contrast to CYP2D6, there are no activity scores for CYP2C19; therefore, establishing a method for including PC of CYP2C19 is more challenging. Currently, there is no consensus about dealing with weak/moderate/strong inhibitors. Prior to including CYP2C19 PC into clinical routine processes, studies must show that the serum concentrations correlate better with the functional enzyme status than with the genotype-inferred phenotype; if relevant, a consensus on how to adjust for PC has to be developed. In the meantime, to ensure an effective and safe phar-

macotherapy in patients affected by CYP2C19 PC and treated with drugs metabolized by CYP2C19, therapy should be closely monitored by TDM to prevent adverse drug reactions.

We explored the association between pharmacokinetics and CYP2C19 phenotypes and functional enzyme status using linear regression analyses to control for age and sex. In analyses on venlafaxine, amitriptyline, and risperidone, we also controlled for CYP2D6 functional enzyme status, as CYP2D6 has previously been shown to impact drug exposure of these drugs [12].

Venlafaxine is primarily metabolized by CYP2D6 and, to a lesser extent, by CYP2C19 [34, 35], making the impact of CYP2C19 alone harder to assess as a single gene. Therefore, for better accuracy, we evaluated the CYP2D6/CYP2C19 combination. CD_{AM} of venlafaxine was not associated with CYP2C19 phenotypes nor with functional enzyme status. This contrasts with initial results that CYP2C19 phenotypes affected the active moiety serum concentration of venlafaxine [22]. However, previously, CYP2C19 was assessed as a single gene, not CYP2D6/CYP2C19 in combination. Thus, the combined approach showed that CYP2D6 rather than CYP2C19 impacted CD_{AM} of venlafaxine (CD_{AM} was associated with CYP2D6 functional enzyme status with higher CD_{AM} in CYP2D6 IM compared to NM (**Supplement 6**)), which is in accordance with PGx dosing guidelines for venlafaxine [4].

According to venlafaxine, in the metabolism of amitriptyline, CYP2D6 is primarily involved and should be considered in combination with CYP2C19 [2]. Therefore, corrected for the CYP2D6 functional enzyme status, CYP2C19 was associated with CD_{AM} of amitriptyline with lower CD_{AM} in RM and UM compared to NM. This concurs with dosing guidelines, considering CYP2D6 and CYP2C19 phenotypes for the treatment with amitriptyline [2]. None of the patients on amitriptyline had been taking medications with relevant inhibition or induction effects on CYP2C19 to cause PC. Therefore, it is not possible to determine the impact of PC.

In our clinical routine setting, we found that CD of sertraline was associated with CYP2C19 phenotypes and functional enzyme status. The number of NM, IM, PM, RM, and UM did not differ when applying $PC_{Bousman}$ and $PC_{Hahn\&Roll}$. This highlighted the major role of CYP2C19 in the metabolism, more precisely in the *N*-demethylation of sertraline *in-vivo*, even if other CYP enzymes are also involved [36–38]. This result supports clinical guidelines giving dosing recommendations based on CYP2C19 phenotypes [1, 4, 39–41].

Additionally, escitalopram is mainly metabolized by CYP2C19 [4]; it has been recommended that in escitalopram-treated patients, CYP2C19 phenotypes should be considered for dose adjustments [4, 41]. This is in accordance with our results that CYP2C19 phenotypes were associated with CD of escitalopram. In addition to these results, *CYP2C19* was associated with serum concentrations below, above or within the therapeutic reference range of escitalopram. Patients with serum concentrations below the therapeutic reference range are more often RM, compared to IM; in contrast patients with serum concentrations within the therapeutic reference range were more often IM than RM. Thus, CYP2C19 RM may have an increased risk for low serum concentrations. However, according to amitriptyline, no patients on escitalopram were taking medications with relevant inhibition or induction effects on CYP2C19 to cause PC.

As serum concentrations of mirtazapine, risperidone, and quetiapine were not associated with CYP2C19 phenotypes/functional enzyme status, we demonstrated that *CYP2C19* does not affect the serum concentrations of these drugs in a clinically relevant way [31]. This is in accordance with the knowledge that CYP2C19 is not involved in the metabolism of these drugs [31]. In consequence, drug-drug interactions with respect to CYP2C19 are likely negligible for mirtazapine, risperidone, and quetiapine.

This shows that enzymes with altered function can possibly be compensated by other enzymes involved in the metabolism of the drug. In consequence, as shown previously for sertraline, a combined pharmacogenomics algorithm including more than two genes may predict the serum concentrations more precisely than one or two individual genes [42]. Bousman, therefore, proposed evidence-based panel testing with a minimum gene set (CYP2C19, CYP2D6, CYP2C9, HLA-A, HLA-B) [43].

Strengths and limitations

The major strength of our analysis is the relevance for a routine clinical setting. Our retrospective naturalistic study in two independent cohorts provides clinical routine real-life data, including a high number of patients. Pharmacokinetic analyses were controlled for age and sex and, if relevant, for the CYP2D6 functional enzyme status. However, due to the limited number of patients who received CYP2C19-affecting drugs and whose phenotype was consequently corrected for PC, it cannot be assessed whether correction for PC and if so, if $PC_{Bousman}$ or $PC_{Hahn\&Roll}$ is better associated with serum concentrations than the genotype-inferred phenotype. Inhibitors and inducers derived from index drugs were categorized as weak/moderate/strong [6]. This categorization of inhibitor/inducer strength, however, is not consistent among different sources. Nevertheless, using the Flockhart table was in line with a previous study by Bousman et al. [7]. Clinical data, for example, clinical improvement, were not available in both cohorts. A limitation of our study is that daily doses of the inhibitors/inducers of CYP2C19 were not recorded due to the retrospective nature of this study. However, a recent study showed that the phenoconversion effect might be dose-dependent [44]. Also, the phenoconversion was calculated based on the genetic phenotype, not on haplotypes due to the low number of patients; however, a study of de Jong showed that the phenoconversion might depend upon the specific polymorphism (e. g., *1/*17 vs. *2/*17) [45]. Moreover, patients were not restricted to a diet, thus, nutrition may have affected enzyme inhibition/induction. Comorbidities and ethnicities were not recorded. Inclusion criteria in both samples were not the same; the Wuerzburg cohort included all patients from which TDM and PGx were available; in contrast, in the Frankfurt cohort, only patients suffering from a depressive episode were included. In addition, drugs are not metabolized by one enzyme but by multiple enzymes in combination; however, we considered only CYP2C19, if relevant, in combination with CYP2D6. Nevertheless, as such real-life data on PGx are rare, our results are important for supporting routine PGx-testing to provide *precision medicine*.

Conclusion

Phenoconversion effects are relevant for CYP2C19; however, occur less often due to the limited use of CYP2C19 perturbating drugs,

compared to CYP2D6. Including PC effects for both enzymes in clinical routine processes may maximize the potential benefits of PGx testing due to an improvement in the prediction of pharmacokinetics, as not only the genotype-inferred phenotype but the more specific (dynamic) functional status of the enzyme is taken into account. However, before including CYP2C19 PC in routine clinical processes, studies with large numbers of patients and sufficient power must show that the serum concentrations correlate better with the functional enzyme status than with the genotype-inferred phenotype. If relevant, a consensus on how to adjust for PC has to be developed. In our study with limited sample size, PC of CYP2C19 changes phenotypes but does not provide superior correlations with serum concentrations. Based on our results, we suggest therapeutic drug monitoring to ensure adequate serum concentrations in individual patients treated with CYP2C19-affecting drugs, for example, esomeprazole and fluoxetine.

Ethical approval

All procedures performed in the analysis involving human participants were in accordance with the ethical standards of the institutional research committees and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Author contributions

Project administration: J. Deckert, M. Scherf-Clavel, and M. Hahn; data collection: M. Scherf-Clavel, A. Eckert, M. Hahn, and A. Frantz; analysis and interpretation of the data: M. Scherf-Clavel; writing—original draft preparation: M. Scherf-Clavel; writing—review and editing: M. Scherf-Clavel, H. Weber, S. Unterecker, J. Deckert, A. Reif, M. Hahn, A. Eckert, and A. Frantz.

All authors have approved of the contents of this manuscript and provided consent for publication.

Conflicts of interest

J. Deckert was a co-recipient of a grant of the Bavarian Ministry of Economic Affairs, Regional Development and Energy (BayMED, MED-1604–0010) and an investigator in a European grant (Horizon 2020 SME program of the European Union ref 696802) to P1Vital. J. Deckert and H. Weber receive funding from the Deutschen Zentrum für Luft- und Raumfahrt (DLR) - Förderkennzeichen 01EK2204G (P4D, Project SP1, SP5A and Coordination). M. Scherf-Clavel, and S. Unterecker have no conflicts of interest. A. Reif has no conflicts of interest with relevance to the present work. M. Hahn, A. Eckert and A. Frantz have no conflicts of interest.

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