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ORIGINAL ARTICLE

Significantly lower CYP2D6 metabolism measured as the *O/N*-desmethylvenlafaxine metabolic ratio in carriers of *CYP2D6*41* versus *CYP2D6*9* or *CYP2D6*10*: a study on therapeutic drug monitoring data from 1003 genotyped Scandinavian patients

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Keywords CYP2D6, *CYP2D6*10*, *CYP2D6*41*, *CYP2D6*9*, genotype, phenotype

AIMS

*CYP2D6*9*, *CYP2D6*10* and *CYP2D6*41* are the most frequent reduced-function *CYP2D6* alleles in Caucasians. Despite lacking *in vivo* evidence, they are collectively classified with an enzyme activity score of 0.5. Thus, the aim of this study was to compare the functional impact of *CYP2D6*9*, *CYP2D6*10* and *CYP2D6*41* on CYP2D6 metabolism in a large patient population.

METHODS

A total of 1003 patients (mainly Caucasians) with data on *CYP2D6* genotype and serum concentrations of venlafaxine and metabolites were included from a therapeutic drug monitoring service in Oslo, Norway. The *O*-desmethyl-to-*N*-desmethyl-venlafaxine metabolic ratio (MR) was applied as CYP2D6 biomarker and compared (Mann–Whitney) between carriers of *CYP2D6*9–10* (merged) and *CYP2D6*41*, either combined with *CYP2D6*1* or non-coding (*null*) alleles. MR subgroup estimates were obtained by multiple linear regression for calculations of *CYP2D6*9–10* and *CYP2D6*41* activity scores.

RESULTS

MR was significantly lower in carriers of *CYP2D6*41* than *CYP2D6*9–10* ($P < 0.002$). The majority of *CYP2D6*41/null* carriers (86.7%) had MR in the observed range of *CYP2D6null/null* carriers compared with the minority of *CYP2D6*9–10/null* carriers (17.4%). *CYP2D6* genotype explained 60.7% of MR variability in the multivariate analysis providing subgroup estimates of 9.54 (95% CI; 7.45–12.20), 3.55 (2.06–6.10), 1.33 (0.87–2.05) and 0.47 (0.35–0.61) in carriers of *CYP2D6*1/null* ($n = 269$), *CYP2D6*9–10/null* ($n = 17$), *CYP2D6*41/null* ($n = 30$) and *CYP2D6null/null* ($n = 95$), respectively. Based on these estimates, the calculated activity score of *CYP2D6*41* was 0.095 compared to 0.34 for *CYP2D6*9–10*.

CONCLUSIONS

CYP2D6 metabolism measured as the *O/N*-desmethylvenlafaxine ratio is significantly lower in Scandinavian carriers of *CYP2D6*41 vs. CYP2D6*9–10*. Thus, these alleles should be differentiated when classifying CYP2D6 phenotype from genotype.

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- CYP2D6 is the most relevant polymorphic drug-metabolizing enzyme.
- CYP2D6 phenotype closely relates to genotype, and allelic activity scores are used when interpreting individual phenotype based on *CYP2D6* genotype.
- The most common *CYP2D6* variant alleles encoding reduced metabolism in Caucasians, i.e. *CYP2D6*9*, *CYP2D6*10* and *CYP2D6*41*, are collectively classified with an activity score of 0.5 without evidence from *in vivo* studies. Thus, it is important to quantify and compare their impact on CYP2D6 phenotype.

WHAT THIS STUDY ADDS

- CYP2D6 phenotype, as measured by the *O/N*-desmethylvenlafaxine metabolic ratio in therapeutic drug monitoring samples from a large Scandinavian population, is significantly lower in carriers of *CYP2D6*41* than *CYP2D6*9–10*.
- The study suggests a residual CYP2D6-metabolizing activity score of 0.095 for *CYP2D6*41* compared with 0.34 for *CYP2D6*9–10*, which illustrates the importance of differentiating between these alleles when studying the effect of *CYP2D6* genotype on pharmacokinetics and therapeutic response of CYP2D6 substrates.
- As >80% of *CYP2D6*41/null* carriers had *O/N*-desmethylvenlafaxine metabolic ratios in the observed range of *CYP2D6null/null* carriers (poor metabolizers), this subgroup is particularly important to be aware of when interpreting CYP2D6 phenotype from genotype.

Introduction

Cytochrome P450 2D6 (**CYP2D6**) is the most relevant polymorphic enzyme involved in drug metabolism. A large proportion of clinically used drugs (>200) are substrates of CYP2D6 [1], and the inherent multimodality in metabolizer phenotype results in a substantial inter-individual variability in exposure and therapeutic response at similar dosing of the affected agents; e.g. many psychotropic drugs, opioid analgesics and beta blockers [1–4]. More than 100 different *CYP2D6* variant alleles have been described, which may cause reduced, absent, increased or unchanged enzyme activity, and their frequencies are often dependent on ethnicity [5].

CYP2D6 metabolizer status is divided into four different phenotype subgroups, i.e. poor metabolizers (PMs), intermediate metabolizers (IMs), extensive (normal) metabolizers (EMs/NMs) and ultrarapid metabolizers (UMs) [6]. When classifying CYP2D6 phenotype from genotype, homozygous carriers of non-coding variant alleles (*null* alleles) are defined as PMs, while carriers of two *reduced-function* alleles or one *reduced-function* and one *null* allele are merged into a common group of IMs [5]. Individuals carrying >2 fully functional gene copies are defined as CYP2D6 UMs, and the remaining, who possess at least one fully functional *CYP2D6* allele, are classified as EMs/NMs [6]. However, except for CYP2D6 PMs, there is substantial variability in CYP2D6 phenotypes within the respective subgroups, and Hertz *et al*. recently reported that the subgroup of IMs probably should be further refined based on *CYP2D6* genotype [7].

In Caucasian populations, *CYP2D6*9*, **10* and **41* are the most common reduced-function variant alleles [5]. These are collectively classified with activity score of 0.5 [2, 5, 8], despite lack of *in vivo* data comparing their quantitative effect on CYP2D6 metabolism. The *CYP2D6*41* allele is of particular interest in Caucasians, due its high allele frequencies in many European countries [9]. An *in vitro* study showed an 85% reduction in enzyme activity encoded by *CYP2D6*41* compared with wild-type *CYP2D6*1* allele [10]. Reports by Abduljalil *et al*. [11] and Gazzaz *et al*. [12] have also indicated a low *in vivo* metabolizer function in *CYP2D6*41* carriers, but these included a limited number of subjects. Studies of sufficient size evaluating the enzyme activity reduction in *CYP2D6*41* carriers are therefore lacking.

CYP2D6 genotyping is increasingly implemented in clinical routine as a tool for assessment of therapeutic failure or start-of-treatment dose estimations of CYP2D6-metabolized drugs [9, 13, 14]. Thus, the accuracy of genotype–phenotype interpretations becomes more crucial, as this has direct implications for patient treatment. Diplotype activity scores are used when interpreting CYP2D6 phenotype from genotype [2, 5, 15, 16], which implicates the clinical relevance of correct allelic activity score assessments.

Assessments of allelic activity scores require a sensitive and specific CYP2D6 metric (biomarker), which is practical to study in large populations. Recently, we showed that the ratio between the *O*- and *N*-desmethylated metabolites of **venlafaxine** is a well-suited CYP2D6 biomarker by detecting genotype-predicted PMs with a specificity and sensitivity of >85% using a predefined cutoff ratio in a naturalistic setting [17]. Venlafaxine undergoes highly specific CYP2D6-mediated *O*-desmethylation as the major metabolic pathway [18], implying that CYP2D6 phenotype also indirectly regulates metabolite formation via the secondary *N*-desmethylation pathway.

As venlafaxine is a commonly used antidepressant with frequent serum concentration monitoring of drug and metabolites, the *O/N*-desmethylvenlafaxine metabolic ratio offers the possibility of evaluating the impact of *CYP2D6* variant alleles on metabolizer phenotype by matching genotype and biomarker data available from therapeutic drug monitoring (TDM). Thus, the aim of the present study was to compare the functional impact of the reduced-function variant alleles *CYP2D6*9*, *CYP2D6*10* and *CYP2D6*41* on CYP2D6 phenotype in a big Scandinavian TDM cohort of venlafaxine-treated patients.

Methods

Patient inclusion

Patients were retrospectively included from a TDM service at the Center for Psychopharmacology, Diakonhjemmet Hospital, Oslo, Norway, if they had performed (i) *CYP2D6* genotyping, and (ii) measured serum concentration(s) of venlafaxine, including *O*- and *N*-desmethylated metabolites, as part of clinical routine. For serum concentration measurements, only patients with a time interval of 10–26 h between last dose intake of venlafaxine and blood sampling for analysis were included.

A search in the TDM database (Swisslab II, Roche Diagnostics, Berlin, Germany) was performed to identify patients fulfilling inclusion criteria during the period from September 2007 to October 2017. Data retrieved from the database search also comprised information about sex, age (at the time of venlafaxine TDM), prescribed daily dose of venlafaxine, and time between last dose intake and blood sampling for serum concentration analysis within the required 10–26 h interval. Although ethnicity was unknown, as this information is not registered and stored during TDM for legal reasons, the population mainly comprised Caucasians from the general ethnic composition in Norway. Information about comedicated drugs was obtained from information provided on the TDM requisition forms, which were reviewed for all patients identified in the database search. Patients concurrently using the potent CYP3A inducers **carbamazepine**, **phenobarbital** or **phenytoin**, or the potent CYP2D6 inhibitors **bupropion**, **fluoxetine**, **levomepromazine** (>50 mg day^{-1}) or **paroxetine**, were excluded.

The study was approved by the Regional Committee for Medical and Health Research Ethics, and the Hospital Investigational Review Board. Ethical approval was given without requirement for patient consent as the study was based on existing data retrospectively retrieved from a routine TDM service.

CYP2D6 genotype

Analyses of *CYP2D6* variant alleles were performed using Taqman-based real-time PCR assays implemented for routine pharmacogenetic analyses at the Center for Psychopharmacology, Diakonhjemmet Hospital. The *CYP2D6* pharmacogenetic panel included the *null* alleles *CYP2D6*3* (*rs35742686*), *CYP2D6*4* (*rs3892097*), *CYP2D6*5* (whole gene deletion) and *CYP2D6*6* (*rs5030655*), the reduced-function variants *CYP2D6*9* (*rs5030656*), *CYP2D6*10* (*rs1065852*) and *CYP2D6*41* (*rs28371725*), as well as copy number analysis to identify multiplication of functional alleles giving rise to ultrarapid metabolism. It has been established that the contribution of unknown rare genetic variants to inter-individual variation in CYP2D6 activity is limited [19], so the genotyping panel covered at least 95% of the variant *CYP2D6* alleles of relevance for CYP2D6 phenotype in the population.

Due to the separately low frequencies of *CYP2D6*9* and *CYP2D6*10*, and initial assessments showing no differences in MR between comparable subgroups ($P > 0.1$; Mann–Whitney), these variants were merged to form a *CYP2D6*9–10* grouping in all further assessments in the study. Prior to statistical analyses, the patients were divided into the following subgroups according to *CYP2D6* genotype: *CYP2D6*1/*1* (absence of variants detected in the assays), *CYP2D6*1/*9–10*, *CYP2D6*1/*41*, *CYP2D6*1/null*, *CYP2D6*9–10/*9–10*, *CYP2D6*9–10/*41*, *CYP2D6*41/*41*, *CYP2D6*9–10/null*, *CYP2D6*41/null*, and *CYP2D6null/null* (i.e. poor metabolizers; PMs). In addition, patients detected with more than two fully functional *CYP2D6* alleles (*CYP2D6*1/*1XN*) were defined as ultrarapid metabolizers (UMs), while those with more than two gene copies and concurrent detection of *null* or *reduced-function CYP2D6* variant alleles were undefined and excluded before statistical analysis.

CYP2D6 phenotype

The **O-desmethyl**-to-*N*-desmethylvenlafaxine (*O/N*-desmethylvenlafaxine) metabolic ratio (MR) was used as CYP2D6 phenotype biomarker to compare the functional impact of *CYP2D6*9–10* and *CYP2D6*41* in the study population. The appropriateness of the biomarker for this purpose was supported by a previous study showing that genotyped PMs were detected with a specificity and sensitivity of 87% and 93%, respectively [17].

Serum concentrations of *O*-desmethyl- and *N*-desmethylvenlafaxine were analysed as a part of TDM of venlafaxine at the Center for Psychopharmacology, Diakonhjemmet Hospital. The analytical method was carried out by use of an ultraperformance liquid chromatography-mass spectrometry (UPLC-MS/MS) assay with inter- and intra-day imprecision and inaccuracy parameters <10% [17].

Statistical analyses

In the initial assessment, unadjusted measurements of *O/N*-desmethylvenlafaxine MRs in *CYP2D6*1/*9–10 vs. CYP2D6*1/*41* carriers, and *CYP2D6*9–10/null vs. CYP2D6*41/null* carriers, were compared by univariate Mann–Whitney tests. Then, a multiple linear regression analysis was performed to provide covariate-adjusted MR estimates of all genotype subgroups. Covariates included in the multiple linear regression analysis were sex, age, prescribed daily dose of venlafaxine, and time between drug intake and blood sampling (within the inclusion criterion interval of 10–26 h). Log-transformed MR values were applied in the multiple linear regression analysis, but the provided genotype subgroup estimates (geometric means) were backtransformed for data presentation and calculation of activity scores of *CYP2D6*9–10* and *CYP2D6*41*.

Statistical analyses were performed using IBM SPSS Software version 22.0 (IBM Corp., Armonk, New York), and *P* values <0.05 defined as statistically significant. GraphPad Prism version 5 (GraphPad Software Inc., La Jolla, California) was used for graphical presentations.

*Calculation of activity scores of CYP2D6*9–10* and *CYP2D6*41*

When calculating the residual enzyme activity scores of *CYP2D6*41* and *CYP2D6*9–10*, the covariate-adjusted MR estimates from the multiple linear regression analysis were applied. Since only the enzyme activity encoded by *CYP2D6null* alleles is certain (0%), the activity scores of

*CYP2D6*9–10* and *CYP2D6*41* were calculated by relating MR estimates in carriers of *CYP2D6*9–10/null* and *CYP2D6*41/null* on a linear scale defining the MR of *CYP2D6null/null* carriers as score '0' and the MR of *CYP2D6*1/null* carriers as score '1'. Hence, the following formula was applied to calculate the respective activity scores (AS) of *CYP2D6*9–10* and *CYP2D6*41*, denominated '**X**':

$$(\text{MR } CYP2D6\mathbf{X}/null - \text{MR } CYP2D6null/null)/(\text{MR } CYP2D6^{*}1/null - \text{MR } CYP2D6null/null) = \text{AS of } \mathbf{X}$$

Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY [20], and are permanently archived in the Concise Guide to PHARMACOLOGY 2017/18 [21].

Results

Study population

After excluding 14 patients concurrently using venlafaxine with either potent CYP2D6 inhibitors (bupropion, n = 5; levomepromazine, n = 4; fluoxetine, n = 2) or CYP3A inducers (carbamazepine, n = 2; phenytoin, n = 1), a total of 1021 patients who had performed both *CYP2D6* genotyping and serum concentration analysis of venlafaxine and metabolites (within the defined time interval of 10–26 h from last dose intake) were included from the TDM database search. Among these, 18 patients were genotyped with more than two gene copies and concurrent detection of *null* (*CYP2D6*4*) or reduced-function (*CYP2D6*41*) variant alleles and excluded before statistical analysis. Thus, 1003 patients were included for comparison of the functional impact of *CYP2D6*9*, *CYP2D6*10* and *CYP2D6*41* on the *O/N*-desmethylvenlafaxine MR.

CYP2D6 genotype distribution. The *CYP2D6* genotype distribution in the population is presented in Table 1. The proportion of *CYP2D6null/null* carriers in the patient population was 9.3% (95% CI 7.7–11.3%). Among the reduced-function variant alleles, *CYP2D6*41* was most commonly detected in the study population (allele frequency 6.6%), and found in combination with *CYP2D6null* alleles in 2.9% of the patients (95% CI 2.1–4.2%).

The proportions of homozygous carriers of *CYP2D6null* alleles and *CYP2D6*10* alleles were both higher than indicated from their respective allele frequencies, and hence not found to be in Hardy–Weinberg equilibrium ($P < 0.05$). For all other variants, no deviation from Hardy–Weinberg equilibrium was detected ($P > 0.1$). All patients with increased copy number of functional *CYP2D6* alleles were triplicate carriers.

*Metabolic ratios in carriers of CYP2D6*41 vs. CYP2D6*9–10*. Figure 1 shows scatterplots of unadjusted MRs in the included patients according to *CYP2D6* genotype. Patients

Table 1

CYP2D6 genotype distribution in the study population (n = 1021)

Genotype	Number of patients	Proportion	95% confidence interval
CYP2D6*1/*1	413	0.4045	0.3748–0.4349
CYP2D6*1/*9	51	0.0500	0.0381–0.0652
CYP2D6*1/*10	9	0.0088	0.0044–0.0169
CYP2D6*1/*41	74	0.0725	0.0580–0.0901
CYP2D6*1/null	269	0.2635	0.2374–0.2914
CYP2D6*9/*9	2	0.0020	<0.0001–0.0076
CYP2D6*10/*10	5	0.0049	0.0017–0.0118
CYP2D69–10/*41	8	0.0078	0.0037–0.0157
CYP2D6*41/*41	7	0.0069	0.0030–0.0144
CYP2D6*9/null	11	0.0108	0.0058–0.0194
CYP2D6*10/null	6	0.0059	0.0024–0.0131
CYP2D6*41/null	30	0.0294	0.0205–0.0418
CYP2D6null/null	95	0.0930	0.0767–0.1125
CYP2D6*1/*1XN	23	0.0225	0.0149–0.0337
CYP2D6*1/*4XN or *1/*41XN[a]	18	0.0176	0.0110–0.0278

[a]Patient subgroup excluded in the study due to unknown identity of the duplicated allele

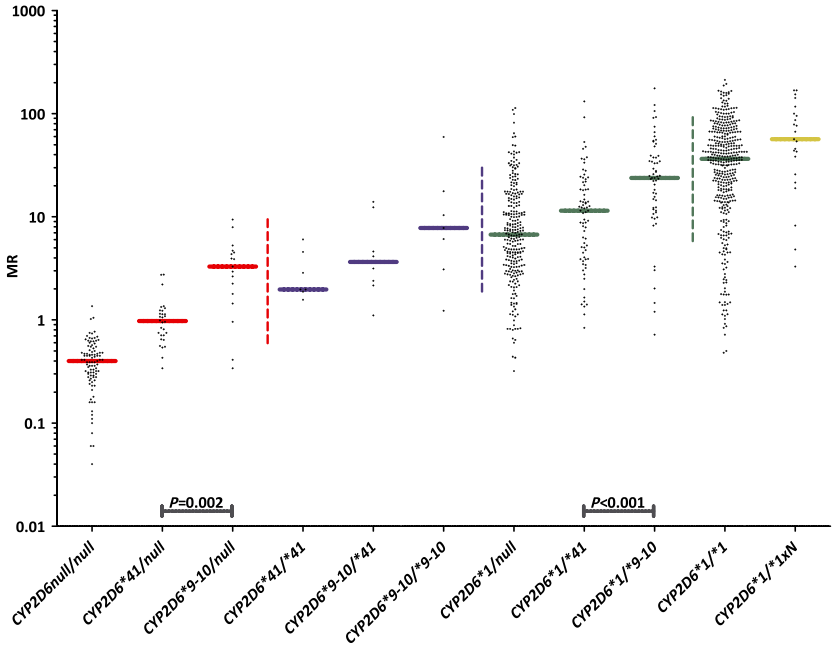

Figure 1

Scatterplots of directly measured, unadjusted, *O/N*-desmethyl-venlafaxine metabolic ratios (MR) in patients with various *CYP2D6* genotypes (lines indicate median values). *P*-values are from Mann–Whitney tests comparing MRs in *CYP2D6*1/*9–10 vs. CYP2D6*1/*41* carriers, and *CYP2D6*41/null vs. CYP2D6*9–10/null* carriers, respectively

with *CYP2D6*1/*41* had significantly lower median MR than *CYP2D6*1/*9* or *CYP2D6*1/*10* carriers ($P < 0.001$), and the same difference was observed for *CYP2D6*41/null vs. CYP2D6*9–10/null* carriers ($P = 0.002$). In *CYP2D6*41/null* carriers ($n = 30$), MRs of 26 patients (86.7%) were in the range of *CYP2D6null/null* carriers compared to only three out of 17 carriers of *CYP2D6*9–10/null* (17.4%) (Figure 2).

In line with the initial univariate statistical analyses, results of the multiple linear regression analysis showed consistently lower covariate-adjusted MR estimates in carriers of *CYP2D6*41* than *CYP2D6*9–10* with comparable allele combinations (Table 2). The covariate-adjusted MR estimate of the *CYP2D6*41/null* subgroup (MR 1.33) was closer to the *CYP2D6null/null* subgroup (MR 0.47) than all other genotypes, and the MR estimate in *CYP2D6*41/*41* carriers was similar to *CYP2D6*9–10/null* carriers (Table 2). Moreover, the covariate-adjusted MR subgroup estimates were similar in patients with *CYP2D6*9–10/*41*, *CYP2D6*9–10/*9–10* or *CYP2D6*1/null* genotypes (Table 2).

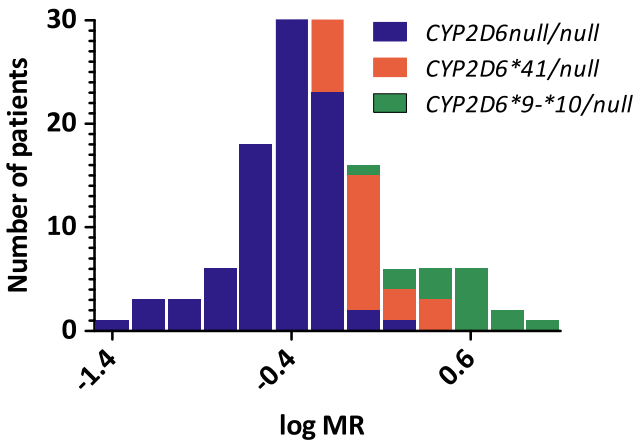

Figure 2

Frequency histogram showing overlaps in CYP2D6 phenotypes, i.e. measured *O/N*-desmethylvenlafaxine metabolic ratios (MR), between patients carrying *CYP2D6null/null*, *CYP2D6*41/null* or *CYP2D6*9–10/null* genotypes

In the multiple linear regression analysis, *CYP2D6* genotype alone explained 60.7% of the variability in MR ($aR^2 = 0.607$) underscoring the suitability of *O/N*-desmethylvenlafaxine as a CYP2D6 phenotype biomarker. When including covariates in the multivariate model (sex, age, venlafaxine dose, and sampling time within 10–26 h after last dose intake), the explained variability in MR was only marginally increased ($R^2 = 0.638$).

*Activity scores of CYP2D6*41* and *CYP2D6*9–10*. When calculating the residual enzyme activity scores of *CYP2D6*41* and *CYP2D6*9–10*, the covariate-adjusted MR estimates in *CYP2D6*41/null* ($n = 30$; MR 1.33) and *CYP2D6*9–10/null* ($n = 17$; MR 3.55) subgroups, respectively, were related those of *CYP2D6null/null* ($n = 95$; MR 0.47), and *CYP2D6*1/null* ($n = 269$; MR 9.54) carriers. On a linear scale defining the MR estimate in *CYP2D6*1/null* carriers as '1' and the MR estimate in *CYP2D6null/null* carriers as '0', the activity score of *CYP2D6*41* was 0.095 (9.5% residual enzyme activity) compared to 0.34 (34% residual enzyme activity) of *CYP2D6*9–10*.

Table 2

Estimated metabolic ratios (MR) of *O*-desmethyl/*N*-desmethyl-venlafaxine in different *CYP2D6* genotype subgroups from multiple linear regression analysis adjusting for covariates (n = 1003). R^2 adjusting for covariates[a] = 0.638; R^2 with CYP2D6 genotype as only independent variable = 0.607

Genotype	MR estimate[b]	95% confidence interval
CYP2D6null/null	0.47	0.35–0.61
CYP2D6*41/null	1.33	0.87–2.05
CYP2D6*9–10/null	3.55	2.06–6.10
CYP2D6*41/*41	3.92	1.75–8.78
CYP2D6*9–10/*41	8.54	3.97–18.38
CYP2D6*9–10/*9–10	9.12	4.07–20.41
CYP2D6*1/null	9.54	7.45–12.20
CYP2D6*1/*41	14.67	10.64–20.22
CYP2D6*1/*9–10	27.16	19.31–38.22
CYP2D6*1/*1	36.83	29.13–46.56
CYP2D6*1/*1XN	68.18	42.21–110.13

[a]Covariate effects: (i) gender +0.16 in MR for males *vs.* females ($P < 0.001$), (ii) time between last dose intake and blood sampling +0.08 in MR per hour from 10–26 h ($P = 0.009$), and (iii) dose −0.015 in MR per 10 mg day^{-1} ($P < 0.001$). No effect of age on MR, included as continuous or grouped ≥65 *vs.* <65 years ($P > 0.7$)
[b]The multiple linear regression analysis was performed with log-transformed MR values, but the tables comprise back-transformed geometric mean estimates with 95% confidence interval (CI)

Discussion

To the best of our knowledge, this is the first study to compare the functional impact of *CYP2D6*9*, *CYP2D6*10* and *CYP2D6*41* on *in vivo* CYP2D6 metabolism. These are the most frequent reduced-function *CYP2D6* variant alleles in Caucasians, and the present study shows that carriers of *CYP2D6*41* consistently exhibit substantially lower CYP2D6 metabolism, as measured by the *O/N*-desmethylvenlafaxine metabolic ratio, than comparable carriers of *CYP2D6*9* or *CYP2D6*10*. While the residual activity score of *CYP2D6*41* was only 9.5%, the score of *CYP2D6*9–10* was approximately three-fold higher. Contrary to current recommendations defining activity scores of all three variants by 0.5 [2, 5, 8], it is therefore crucial to differentiate between these alleles when interpreting CYP2D6 phenotype from genotype.

The low activity score of *CYP2D6*41* calculated in the present study of mainly Caucasians is supported by previous reports by Abduljalil *et al*. [11] and Gazzaz *et al*. [12]. However, these prior studies included a very limited number of *CYP2D6*41* carriers (nine in total), with only one [11] and two [12] subjects being *CYP2D6*41/null* carriers, respectively. In comparison, the present study comprised a large number of patients carrying the *CYP2D6*41/null* genotype (n = 30). Among *CYP2D6*41/null* carriers in our study, more than 80% displayed *O/N*-desmethylvenlafaxine metabolic ratios within the observed range *CYP2D6null/null* carriers, while this was the case for <20% of *CYP2D6*9–10/null* carriers. This is in line with the greater impact of *CYP2D6*41* than *CYP2D6*9–10* on CYP2D6 phenotype, which should provide a basis for discussing the current genotype-based classification of CYP2D6 IMs.

Current guidelines merge genotypes homozygous for any reduced-function alleles, as well as genotypes comprising one reduced-function and one null allele, into a common group of IMs [6]. However, the covariate-adjusted MR subgroup estimates of the present study indicate that carriers of *CYP2D6*41/null* may represent an intermediate-to-poor metabolizing (IPM) phenotype, while carriers of *CYP2D6*9–10/null* or *CYP2D6*41/*41* generally display lower CYP2D6 metabolism than carriers of *CYP2D6*9–10/*41*, *CYP2D6*9–10/*9–10* or *CYP2D6*1/null*. Thus, more refined genotype-based IM subgroup classifications should be considered in future studies investigating the effect of *CYP2D6* genetics on pharmacokinetics or clinical outcomes of candidate drugs; e.g. the prodrug **tamoxifen**, where CYP2D6 metabolizer phenotype could be of critical importance for the therapeutic response [22–25].

Mutations described by Raimundo *et al*. in 2000 led to the identification of the *CYP2D6*41* variant allele [26], which is believed to result in reduced enzyme expression due to a splicing defect [10, 27]. Our study consistently shows that carriers of *rs28371725* (*c.985 + 39G>A*; denoted as '*CYP2D6*41*') exhibit significantly lower CYP2D6 metabolism, as measured by the *O/N*-desmethylvenlafaxine metabolic ratio, than carriers of *CYP2D6*9* or *CYP2D6*10*. Thus, *rs28371725* in *CYP2D6*41* is clearly a marker of the substantially reduced CYP2D6 metabolism in our study population, and possibly causative for the decreased expression of *CYP2D6*41* by producing a splicing defect [10, 27]. However, it could not be excluded that other mutations in linkage disequilibrium with *CYP2D6*41* may play a role in the phenotype effect, as indicated in a study by Wang *et al*. [28]. Furthermore, our population comprised Scandinavians of mainly Caucasian origin, implying that the calculated *CYP2D6*41* activity score might be different in other populations due to interethnic haplotype variability. The same point also applies for *CYP2D6*9–10*, and another aspect is that the impact of *CYP2D6*41 vs. CYP2D6*9–10* on the metabolic profile of other drugs than venlafaxine could be different. Therefore, it is important to further investigate the relative impact of these variant alleles for CYP2D6 metabolism in various ethnic groups, focusing on the potential relevance of haplotype differences and substrate diversity in large study populations.

The *O/N*-desmethylvenlafaxine MR was recently shown to have a high specificity and sensitivity (>85%) in detecting genotype-predicted CYP2D6 PMs [17]. In the present study on more than 1000 patients, *CYP2D6* genotype alone explained 60.7% of the variability in the MR when accounting for significant covariates in the multiple linear regression analysis. As *CYP2D6* genotype and phenotype is known to be closely correlated, this underscores the suitability of *O/N*-desmethylvenlafaxine MR as a CYP2D6 biomarker for comparing the functional impact of different variant alleles. The prescribed venlafaxine dose and sampling time

point (within 10–26 h) only had minor quantitative effects on the MR, indicating fairly similar relative formation by dose and elimination kinetics of the two venlafaxine metabolites.

Despite highly significant MR differences between the genotype subgroups, there was also considerable intra-subgroup variability in phenotypes, probably reflecting a combination of additional genetic and non-genetic factors affecting venlafaxine metabolism. Some of the variability was likely due to the presence of *CYP2D6* variants not included in the genotyping panel of the study, e.g. *CYP2D6*35*, which has been associated with increased CYP2D6 metabolism [29]. Another variant not included in the panel was *CYP2D6*2*, an allele generally assigned to encode 'normal' enzyme activity [30], but where some studies have reported a reduced CYP2D6 phenotype as well [11]. In a recent review article by Gaedigk *et al*., it was further pointed out that other sources than *CYP2D6* genotype likely contribute to the variability in CYP2D6-mediated drug metabolism [15]. While *O*-desmethylation of venlafaxine is CYP2D6-specific, **CYP3A4** and **CYP2C19** are both involved in the *N*-desmethylation pathway [31]. Thus, individual differences in CYP3A4 and/or CYP2C19 metabolism may also to some extent contribute to the intra-subgroup MR variability. Furthermore, the individual differences in activities of the enzymes involved in the *N*-desmethylation pathway of venlafaxine may potentially limit the linear MR scaling over a large interindividual range when calculating the *CYP2D6* alleles' activity scores.

The present study has some limitations related to the naturalistic design and heterogeneous nature of included patients. Although the TDM requisition forms were thoroughly reviewed to identify and exclude users of potent CYP2D6 inhibitors or CYP3A4 inducers, the comedication profiles written on the forms might be incomplete, implying that some patients were using drugs (or herbal agents) affecting the MR. Another issue was that ethnicity of the patients was not available from the TDM records. Despite the majority being Caucasians, the deviation of *CYP2D6*10* from Hardy–Weinberg equilibrium likely reflects an ethnic heterogeneity of the population, which was supported by name attributes on the TDM requisition forms indicating Asian descent of the three homozygous carriers of *CYP2D6*10*. Regarding the higher frequency of patients carrying the *CYP2D6null/null* genotype, i.e. 9.3%, compared to healthy Caucasians [1, 4, 32], a likely explanation is patient inclusion from a naturalistic TDM setting, where cases with therapeutic problems and a poor metabolizer phenotype might be overrepresented. Thus, it is important to be aware that the detected frequency of *CYP2D6*41/null* carriers in the study population (2.9%) is probably higher than in Caucasian populations in general.

In conclusion, this study shows that CYP2D6 metabolism measured as the *O/N*-desmethylvenlafaxine ratio is significantly lower in Scandinavian carriers of *CYP2D6*41* than *CYP2D6*9–10*. Our findings in a large population suggest that the residual enzyme activity score of *CYP2D6*41* is only 9.5% of normal compared to 34% for *CYP2D6*9–10* in subjects of mainly Caucasian origin. Thus, these variant alleles should be differentiated when classifying CYP2D6 phenotype from genotype.

Contributors

All the authors contributed to the study design. T.H., M.J. and E.M. performed the research. T.H. and E.M. wrote the manuscript with input/modifications from all the other authors. All authors reviewed the manuscript and approved the final version.

Competing Interests

There are no competing interests to declare.

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