Evaluation of the CYP2D6 Haplotype Activity Scores Based on Metabolic Ratios of 4,700 Patients Treated With Three Different CYP2D6 Substrates

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The metabolic activity of the polymorphic CYP2D6 enzyme is dependent on the CYP2D6 genotype; however, the guidelines for translating the genotype into phenotype, which are of relevance for adequate drug dose personalization, are ambiguous. In the present study, retrospective therapeutic drug monitoring data from 4,700 CYP2D6 genotyped patients treated with risperidone, venlafaxine, and/or aripiprazole were analyzed to quantify the effect of CYP2D6 genotype on the CYP2D6 metabolic activities, as measured by metabolic ratios of these substrates. The patients were categorized into diplotypes based on the presence of normal function (CYP2D6Norm), nonfunctional (CYP2D6Nonf), and decreased function (CYP2D6Decr; i.e., CYP2D6*9, CYP2D6*10, and CYP2D6*41) CYP2D6 haplotypes. Significant correlations between the metabolic ratios were observed in patients (n = 77–103) cotreated with risperidone and venlafaxine, risperidone and aripiprazole, or venlafaxine and aripiprazole (ρ = 0.874, 0.785, and 0.644, respectively; P < 0.001 for all). Relative metabolic CYP2D6 diplotype activity was calculated based on that the metabolic ratios, where median values for CYP2D6Nonf/Nonf and CYP2D6Norm/Norm subgroups were set to 0% and 100%, respectively. The relative CYP2D6 activities were: 7.0% for CYP2D6Nonf/*41, 16.7% for CYP2D6Nonf/*9–10, 13.2% for CYP2D6*41/*41, 24.9% for CYP2D6*41/*9–10, 33.1% for CYP2D6*9–10/*9–10/Nonf/Norm, 41.3% for CYP2D6*9–10/Norm, and 149.2% for CYP2D6Norm/Normx2. Compared with the CYP2D6Norm alleles, the activity scores of CYP2D6*41 and CYP2D6*9–10 alleles were estimated to be one sixth and one third, respectively. The results of this highly powered study provide a solid basis for the translation of the CYP2D6 genotype into a drug metabolic phenotype.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ The guidelines for genotype-to-phenotype translations of different CYP2D6 diplotype carriers is to a great extent based on arbitrary values, which is particularly pronounced for variant haplotypes encoding decreased CYP2D6 activity.

WHAT QUESTION DID THIS STUDY ADDRESS?

☑ Using a large therapeutic drug monitoring based cohort based on 4,700 patients treated with 3 different CYP2D6 substrates, the relative metabolic CYP2D6 activity of patients carrying different CYP2D6 diplotypes was addressed based on the drug metabolic ratios.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

☑ An activity-based table for estimation of CYP2D6 diplotype based on risperidone, venlafaxine, and aripiprazole is presented and activity scores of the decreased-function alleles, compared with normal-function CYP2D6Norm allele, are estimated to one sixth (~18%) for the CYP2D6*41 and one third (~34%) for the CYP2D6*9 and CYP2D6*10 alleles.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

☑ The results provide a more precise basis for the translation of the CYP2D6 genotype into a drug metabolic phenotype and are expected to improve the implementation of the CYP2D6 pharmacogenetic analyses into the clinical settings.

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The polymorphic cytochrome P450 2D6 (CYP2D6) enzyme metabolizes about 30% of clinically used drugs and the CYP2D6 gene is one of the most polymorphic genes in the entire human genome. The alleles encoding normal functional metabolic activity (CYP2D6Norm) mainly encompass the common CYP2D6*1 and CYP2D6*2 alleles, whereas numerous common and rare alleles are associated with no function (CYP2D6Nonf) or substantial decrease of the metabolic activity (CYP2D6Decr). In addition; copy number variations are seen in the locus, causing, for example, poor or ultrarapid metabolism. However, although significant progress has been made in the CYP2D6 genetic research, translation of the genotypes into phenotypic categories has been quite problematic. Traditional categorization stratifies the patients into 4 metabolizer categories: CYP2D6 poor metabolizers (PMs), intermediate metabolizers (IMs), normal metabolizers (NMs), and ultrarapid metabolizers (UMs); however, the phenotypic categorization is not as straightforward, with respect to CYP2D6 diplotype. It is generally accepted that the carriers of the CYP2D6Norm/Norm diplotype are NMs, CYP2D6Norm/Normx2 carriers (gene duplication) are UMs, CYP2D6Nonf/Decr are IMs, and CYP2D6Null/Null are PMs. However, the position of patients with CYP2D6Norm/Nonf, CYP2D6Norm/Decr, and CYP2D6Decr/Decr in such a categorization has been a matter of debate for a long time and has differed among the relevant sources. Recently published consensus recommendations on standardizing CYP2D6 genotype-to-phenotype translations published by the Clinical Pharmacogenetics Implementation Consortium (CPIC) and Dutch Pharmacogenetics Working Group (DPWG) represent an important step in resolving these discrepancies. The classification in these consensus guidelines is, however, based on metabolic activity scores for individual CYP2D6 alleles than on a very strict categorization system. The CYP2D6Norm allele exhibits an activity score of 1, because it possesses 100% metabolic activity; the CYP2D6Null allele has an activity score of 0, due to the complete loss of enzyme; and the CYP2D6Decr alleles encode enzyme activities in between CYP2D6Norm and CYP2D6Nonf. Based on limited data from in vivo studies, the consensus guidelines have estimated the common CYP2D6Decr alleles CYP2D6*41 and CYP2D6*9 with 50% residual metabolic activity, whereas CYP2D6*10 is estimated with 25% residual metabolic activity.

However, several open questions remain unanswered: (i) can metabolic activity of a diplotype be universally defined for all the drugs or is it substrate-specific; and, if yes, to what degree? (ii) Can metabolic activity of a diplotype be calculated by simply summing up the activity scores of two (or more, in case of gene duplication) alleles or the calculation is more complex? (iii) Are the estimates for metabolic activity for the CYP2D6Decr, in particular for the common CYP2D6*9, CYP2D6*10, and CYP2D6*41 alleles, precise enough? This study aims to answer these questions by analyzing the therapeutic drug plasma level monitoring (TDM) and CYP2D6 genotype data from 4,700 unique patients treated with risperidone, venlafaxine, and or aripiprazole; these CYP2D6 substrates exhibit very different affinities for the enzyme and half-lives of 3, 5, and 75 hours, respectively.

**METHODS**

Patient data were retrospectively obtained from a routine TDM database at the Center for Psychopharmacology, Diakonhjemmet Hospital, Oslo, Norway, for the period between January 1, 2005, and December 31, 2019. The total of 12,395 TDM entries of 4,700 unique patients treated with risperidone, venlafaxine, and/or aripiprazole were initially obtained as 3 separate databases; one for each drug. All patients were Norwegian inhabitants, confirmed by a unique social security number (patient ID), and most (>95%) were assumed to be of Scandinavian ancestry based on the ethnic composition of the general Norwegian population. The presence of other ethnicities could not be drawn from the requisition form, due to ethical reasons and risk of personal identity breach. On the basis of information provided on the database requisition forms, median age was 38, 46, and 33 years; outpatients represented 62%, 82%, and 69%; women represented 51%, 63%, and 53% of risperidone-treated, venlafaxine-treated, and aripiprazole-treated patient cohorts, respectively. The use of anonymized patients’ data for the purpose of this study was approved by the Regional Committee of South-Eastern Norway for Medical and Health Research Ethics and the Hospital Investigational Review Board.

The patients were genotyped as a part of the routine clinical care for the common CYP2D6Norm, including CYP2D6*4 (rs35742686), CYP2D6*4 (rs3892099), CYP2D6*5 (whole gene deletion), CYP2D6*6 (rs5030655), and CYP2D6Decr, including CYP2D6*9 (rs5030656), CYP2D6Decr*10 (rs1065852), and CYP2D6*41 (rs28371725) alleles; in addition, the gene copy number was determined in order to detect the whole gene deletion or multiplication in accordance with manufacturer’s protocol (Assay ID: Hs00010001_en; Thermo Fisher Scientific, Waltham, MA). If a variant allele was not detected, the allele was considered to be connected with normal metabolic activity (CYP2D6Norm). The ultraperformance liquid chromatography-tandem mass spectrometry methods for quantifications of the CYP2D6 substrate drugs risperidone, venlafaxine and aripiprazole, and their respective metabolites (i.e., 9OH-risperidone, O-desmethylvenlafaxine, and dehydroaripiprazole), have previously been described in detail. All the calibration curves were linear (R^2 > 0.99), whereas imprecision and inaccuracy parameters of the assays were lower than 5% for the validated ranges. For the quantification of CYP2D6 metabolic activity, the ratios between metabolite and parent compound were used; in particular, (9OH-risperidone)/(risperidone), (O-desmethylvenlafaxine)/(venlafaxine), and (dehydroaripiprazole)/(aripiprazole).

The entries from datasets of the 3 different CYP2D6 substrates were initially tested for inclusion; an entry was excluded from the analysis if (i) a long acting dosage forms were used, because the constant parent compound efflux into the circulation would affect metabolic ratios, (ii) the time between the last dose intake and sample withdrawal time was between 10 and 30 hours, to minimize the confounding of the non-steady state kinetics, and (iii) parent drug or metabolite was not detected in the plasma (Figure 1). To address the similarity between the metabolic ratios of different drugs in prediction of CYP2D6 metabolic activity, which arose from interindividual variability in drug metabolism, Spearman’s correlation test was performed between metabolic ratios of two drugs for the patients cotreated with two of the analyzed drugs. The entries were included in the correlation analysis if the “Patient ID” and “TDM date and time” were the same in the databases dealing with the patients treated with 2 analyzed drugs; 102 entries with venlafaxine and risperidone cotreatment from 52 unique patients, 103 entries for venlafaxine and aripiprazole treatment from 66 unique patients, and 77 entries for risperidone and aripiprazole treatment from 47 unique patients were included in the analysis.

To quantify the effect of CYP2D6 genotype on CYP2D6 metabolic activity, the patients were categorized based on the CYP2D6 genotype into 11 diplotypes. Because the initial screening analysis demonstrated that the effect of CYP2D6*9 and CYP2D6*10 alleles on metabolic activity are
Figure 1. CONSORT flow diagram of included/excluded patients and entries. Boxes depicting the patients/entries obtained from the database and patients/entries used for the analysis are shaded in gray. Entries were removed based on the following exclusion criteria: (a) long-acting dosage forms were used, (b) the time between the last dose intake and sample withdrawal time was between 10 and 30 hours, (c) parent drug or metabolite was not detected in the plasma, (d) the patients were cotreated with a known CYP2D6 inhibitor, or (e) the categorization was not possible due to inconclusive genotyping. If all the entries for an individual patient were excluded, this meant that the patient was excluded from the analysis. Dose, withdrawal time, and age are represented as median (interquartile range). TDM, therapeutic drug monitoring.

similar (Table S1), these 2 alleles were pooled together into a composite CYP2D6*9–10 haplotype. Prior to the statistical analysis and between-group comparisons, the additional entries were removed from databases if (iv) the patients were cotreated with a known CYP2D6 inhibitor (buproprion, fluoxetine, paroxetine, fluvoxamine, carbamazepine, phenobarbital, phenytoin, levomepromazine), to avoid phenocconversion and if (v) the categorization was not possible due to inconclusive genotyping to avoid misclassification; for example, if the patient was heterozygous and had three CYP2D6 copies, it was unclear which allele is duplicated. For the purpose of pooling the results from all the patients, the metabolic ratio (MR) data was harmonized according to the formula (MR – a)/(b-a); a = median MR for patients with CYP2D6Nonf/Nonf and b = median MR for patients with CYP2D6Norm/Nonf treated with the respective drug. The CYP2D6Norm/Nonf median value was therefore set to 100%, and the CYP2D6Nonf/Nonf median value to 0% metabolic activity. Between-drug comparison for each CYP2D6 diplotypes and a follow-up false discovery rate corrected between-group comparisons were made by the Kruskal–Wallis test. The difference between individual groups was considered nominally significant if the P value obtained by the post hoc test was lower than 0.05, and statistically significant only if it stayed lower than 0.05 after the false discovery rate correction for multiple comparisons. To approximate the ratio of means between regression coefficients for these respective alleles and regression coefficient for CYP2D6Norm, according to the previously published formula.

RESULTS

Initially, the similarity among MRs of risperidone, venlafaxine, and aripiprazole as estimates of CYP2D6 metabolic activity, which occurred due to interindividual variability in drug metabolism, was evaluated. A correlation analysis was performed among the metabolic ratios for the patients cotreated with two of three drugs mentioned (Figure 2) and significant correlations were observed between the MRs of risperidone and venlafaxine (Figure 2a; \( \rho = 0.874 \), \( P < 0.001 \)), venlafaxine and aripiprazole (Figure 2b; \( \rho = 0.644 \), \( P < 0.001 \)), and risperidone and aripiprazole (Figure 2c; \( \rho = 0.785 \), \( P < 0.001 \)). Noteworthy, the distribution of MR values for risperidone and venlafaxine was more spread compared with the narrow distribution of the MR values for aripiprazole (Figure 2).
differentiate between CYP2D6*9 (score 0.5) and CYP2D6*10 (score 0.25), an initial analysis in the current patient material indicated that the effect of the CYP2D6*9 and CYP2D6*10 haplotypes on MRs for all analyzed drugs can be considered equivalent (Table S1) and these 2 haplotypes were therefore merged into a composite CYP2D6*9–10 haplotype for the purpose of the analysis. The CYP2D6 genotype significantly affected MRs for all three drugs (Figure 3) and the haplotype-induced changes were most pronounced regarding risperidone MRs (Figure 3a), somewhat less on venlafaxine metabolic ratio (Figure 3b), and the least on aripiprazole metabolic ratio (Figure 3c). The CYP2D6Nonf/Nonf, CYP2D6Norm/Nonf, and CYP2D6Norm/ Norm subgroups encompassed large numbers of subjects and exhibited profoundly different MRs for all drugs analyzed; therefore, these three diplotypes were set as reference points for all between-diplotype comparisons. For all drugs analyzed, the CYP2D6Null/*41 diplotype group exhibited lower MRs compared with the CYP2D6Norm/Nonf and CYP2D6Norm/Norm subgroups, whereas the CYP2D6*9–10/*9–10 and CYP2D6*41/*41 diplotype exhibited lower MR compared with CYP2D6Norm/Nonf. In addition, CYP2D6Norm/ Normx2, CYP2D6Norm/*41, and CYP2D6Norm/*9–10 had higher values compared with CYP2D6Norm/Nonf. Many other drug-specific significant changes were observed between the different diplotypes, as represented in detail in the Figure 3 and in the Supplementary Tables S2 and S3.

In order to approximate relative metabolic activities (activity scores), the MRs for each drug were harmonized by setting the median MR of patients with CYP2D6Nonf/Nonf to 0% and CYP2D6Norm/Norm to 100% metabolic activity (Figure 4). The analysis of pooled patient data across the 3 CYP2D6 substrates showed that the metabolic activities were: 7.0% for CYP2D6Norm/*41, 16.7% for CYP2D6Nonf/*9–10, 13.2% for CYP2D6*41/*41, 24.9% for CYP2D6*9–10/*9–10, 33.1% for CYP2D6*9–10/*9–10, 41.3% for CYP2D6Nonf/Nonf, 55.0% for CYP2D6*9–10/*9–10, and 149.2% for CYP2D6Norm/Norm (Figure 4, Table 1). Compared with all the diplotypes, the functional alleles exhibited significantly higher metabolic activity compared with CYP2D6Nonf/Nonf diplotype and all diplotypes with less than two CYP2D6Norm alleles exhibit decreased metabolic activity compared with CYP2D6Norm/Norm diplotype (Table S3). None of the diplotypes with metabolic activity higher than CYP2D6Nonf/Nonf and lower than CYP2D6Norm/Norm was statistically distinct from all other such diplotypes, whereas the increase in CYP2D6Norm/Normx2 diplotype, compared with CYP2D6Norm/Norm, did not reach statistical significance (Table S3). The statistical report presenting all between-diplotype comparisons is available in the Supplementary Table S3. Between-drug comparisons indicated that metabolic activity for CYP2D6Nonf/*41 and CYP2D6Norm/Nonf was higher in the patients treated with aripiprazole, compared with the ones treated with risperidone and venlafaxine, whereas the between-drug differences were not detected for other diplotypes (Table S4). Taken together, the difference in metabolic activity between CYP2D6 diplotyes is very pronounced and it is approximated by the MRs of risperidone, venlafaxine, and aripiprazole in a similar but not exactly the same fashion.
To quantify the activity scores of CYP2D6*41 and CYP2D6*9–10, compared with CYP2D6Norm, regression equations were calculated to predict metabolic activity. The numbers of CYP2D6Norm, CYP2D6*41, and CYP2D6*9–10 alleles were analyzed as predictors of the diplotype activity score, whereas the CYP2D6Nonf/Nonf subgroup was considered as the reference (const.).

Constant – Regression equation intercept for zero values of all independent variables

\[ \text{Activity score} = \text{antConst} + B_\text{Dose} \times \text{Dose} \left( \frac{\text{mg}}{\text{day}} \right) + B_\text{Time} \times \text{time} (\text{hours}) + B_\text{Norm} \times n_\text{Norm} + B_\ast41 \times n_\ast41 + B_\ast9–10 \times n_\ast9–10 \]

Constant – Regression equation intercept for zero values of all independent variables

\[ B_\text{Dose} \] – Coefficient for dose; dose (mg/day) – daily dose

\[ B_\text{Time} \] – Coefficient for withdrawal time; time (hours) – time between drug intake and blood sampling

\[ B_\text{Norm} \] – Coefficient for CYP2D6Norm; \( n_\text{Norm} \) – number of present CYP2D6Norm alleles

\[ B_\ast41 \] – Coefficient for CYP2D6*41; \( n_\ast41 \) – number of present CYP2D6*41 alleles

\[ B_\ast9–10 \] – Coefficient for CYP2D6*9 and CYP2D6*10; \( n_\ast9–10 \) – number of present CYP2D6*9 or CYP2D6*10 alleles

The equations were calculated for datasets corresponding to the patients treated with each analyzed drug separately, as well as for the composite dataset, which comprises all the patients; the regression coefficients are presented in Table 1. According to regression equations, the contributions of CYP2D6*41 and CYP2D6*9–10 alleles to metabolic activity are approximately equal to 18% and 34% compared with CYP2D6Norm, respectively (Figure 4, Table 2).

**DISCUSSION**

By studying a large number of subjects, the effect of CYP2D6 genotype on CYP2D6 metabolic activity was quantified at a high precision and statistical power. It was demonstrated that (i) the MRs...
of 3 CYP2D6 substrates with very different half-lives are similar within the same patients and all can predict CYP2D6 metabolic activity in a similar, but not in exactly the same way, (ii) the metabolic activity for each CYP2D6 diplotype represents the sum of haplotype metabolic activities to a very solid degree, and (iii) the activity score of the CYP2D6*41 allele is approximately one-sixth, whereas activity scores of the CYP2D6*9 and CYP2D6*10 alleles are approximately one-third, compared with the activity score of CYP2D6Norm allele. These results are expected to improve the current activity score classifications of decreased-function CYP2D6 variant alleles and the respective CYP2D6 metabolizer categorization.

The intra-individual correlation observed between MRs for these 3 very different substrates argue in favor that the CYP2D6-mediated component of metabolic reaction can be translated from one substrate to another to a reasonable degree. However, the substrate specificity cannot be excluded based on the results on only 3 CYP2D6 substrates and further studies across a wider range of substrates are necessary to adequately test the hypothesis that the CYP2D6 genotype-defined metabolic activity is universal. Noteworthy, whereas the correlation of MRs and similarity in metabolic activity profile of CYP2D6 diplotypes between risperidone and venlafaxine are remarkably high, there are certain discrepancies when the results obtained for the lower affinity substrate aripiprazole are compared with the results obtained for the two high-affinity substrates. The observed discrepancies, however, most likely occurred due to low CYP2D6 affinity and long aripiprazole half-life; in particular, unlike risperidone and venlafaxine metabolism, aripiprazole metabolism is not so much dependent on CYP2D6 catalyzed reactions and, consequently, the variability of
| CYP2D6 genotype | Risperidone | Venlafaxine | Aripiprazole | All drugs | Predicted activity |
|-----------------|------------|------------|-------------|-----------|-------------------|
|                 | N | Nom. | Harm. | N | Nom. | Harm. | N | Nom. | Harm. | N | Harm. | Current | Proposed |
| Nonf/Nonfl      | 70 | 0.34 | (0.29–0.37) | 0.0 | (−0.4–0.3) | 139 | 0.40 | (0.30–0.46) | 0.0 | (−1.7–1.1) | 82 | 0.18 | (0.17–0.18) | 0.0 | (−3.8–3.9) | 291 | 0.0 | (−0.5–0.7) | 0 | 0 |
| Nonf/*41        | 35 | 0.88 | (0.74–1.00) | 4.0 | (2.9–4.9) | 56 | 0.78 | (0.69–0.92) | 6.8 | (5.2–9.3) | 42 | 0.22 | (0.20–0.24) | 16.8 | (9.2–26.4) | 133 | 7.0 | (5.2–8.9) | 25 | 8.3 |
| Nonf/*9-10      | 19 | 2.75 | (1.45–3.86) | 17.7 | (8.2–25.8) | 30 | 1.11 | (1.01–1.14) | 12.8 | (11.0–18.1) | 22 | 0.24 | (0.20–0.28) | 28.2 | (8.9–44.9) | 71 | 16.7 | (12.0–21.5) | 12.5–25 | 16.7 |
| *41/*41         | 10 | 1.29 | (0.63–2.17) | 7.0 | (2.2–13.4) | 10 | 1.31 | (0.92–2.27) | 16.3 | (9.3–33.4) | 11 | 0.23 | (0.16–0.30) | 21.4 | (−7.3–51.4) | 31 | 13.2 | (6.7–20.2) | 50 | 16.7 |
| *41/*9-10       | 5 | 2.82 | (0.88–7.50) | 18.2 | (4.0–52.4) | 12 | 1.83 | (1.34–2.51) | 25.6 | (16.8–37.6) | 5 | 0.27 | (0.17–0.30) | 38.0 | (−0.5–54.0) | 22 | 24.9 | (16.8–38.0) | 37.5–50 | 25 |
| *9-10/*9-10     | 6 | 2.53 | (0.76–17.00) | 16.0 | (3.1–122.0) | 18 | 2.43 | (0.95–3.48) | 36.2 | (9.9–55.0) | 15 | 0.26 | (0.24–0.30) | 35.3 | (−25.3–53.7) | 39 | 33.1 | (18.8–47.3) | 25–50 | 33.3 |
| Nonf/Norm       | 294 | 5.36 | (4.78–6.00) | 36.7 | (32.5–41.5) | 487 | 2.40 | (2.25–2.57) | 35.7 | (33.0–38.7) | 323 | 0.32 | (0.30–0.33) | 59.5 | (50.3–66.7) | 1104 | 41.3 | (38.3–43.6) | 50 | 50 |
| *41/Norm        | 91 | 7.14 | (5.65–9.00) | 49.8 | (38.9–63.4) | 160 | 3.11 | (2.45–3.58) | 48.5 | (36.7–59.6) | 110 | 0.33 | (0.30–0.37) | 64.7 | (50.3–81.4) | 361 | 55.0 | (48.2–59.7) | 75 | 58.3 |
| *9-10/Norm      | 47 | 7.33 | (5.67–11.17) | 51.2 | (39.0–79.3) | 85 | 3.71 | (3.17–4.82) | 59.2 | (49.5–79.0) | 54 | 0.32 | (0.30–0.35) | 62.2 | (53.6–72.3) | 186 | 58.9 | (52.1–68.3) | 62.5–75 | 66.7 |
| Nom/Norm        | 383 | 14.00 | (12.00–16.00) | 100.0 | (85.4–114.6) | 707 | 6.00 | (5.66–6.41) | 100.0 | (93.9–107.4) | 508 | 0.41 | (0.39–0.43) | 100.0 | (92.1–105.3) | 1598 | 100.0 | (95.5–103.7) | 100 | 100 |
| Nom/Norm x 2    | 25 | 28.00 | (16.00–45.00) | 202.5 | (114.6–326.9) | 38 | 9.53 | (7.48–11.96) | 163.0 | (126.3–206.3) | 27 | 0.42 | (0.28–0.53) | 103.1 | (42.9–147.2) | 90 | 149.2 | (114.6–173.3) | 150 | 150 |

All nominal values (Nom.) represent the median values of metabolic ratios ± 95% confidence intervals. Harmonized values (Harm.) are presented as percentages ± 95% confidence intervals and they represent the data transformed in a way that the CYP2D6 Norm/Norm median value is set to 100%, while the CYP2D6 Nonf/Nonfl median value to 0%. Harmonized values are presented for each drug separately and also for the admixed groups of the patients. Predicted activity column represents the expected values for harmonized metabolic ratios in percentages according to the currently available guidelines (Caudle et al.; CYP2D6 Nonf = 0%, CYP2D6 *41 = 50%, CYP2D6 *9 = 50%, CYP2D6 *10 = 25%, and CYP2D6 Norm = 100%) and the guidelines suggested here (CYP2D6 Nonf = 0%, CYP2D6 *41 = 16.7%, CYP2D6 *9-10 = 33.3%, and CYP2D6 Norm = 100%). IM, intermediate metabolizer; IM+, new suggested intermediate-plus metabolizer category, which is placed in between normal and intermediate metabolizers; NM, normal metabolizer; PM, poor metabolizer; UM, ultrarapid metabolizer.
CYP3A4-mediated metabolism may significantly modulate the aripiprazole metabolic ratio. Therefore, although the results indicate that the CYP2D6 genotype-encoded change in CYP2D6 metabolic activity is somewhat higher for high-affinity substrates, the effect of diplotypes on CYP2D6 MRs can be considered relatively consistent among the 3 analyzed substrates. Importantly, the clinical relevance of CYP2D6 genotype and CYP2D6 metabolism is substrate dependent; it is determined by the relative importance of CYP2D6-mediated and alternative metabolic pathways in substrate metabolism and by the pharmacological properties of the substrate and metabolite(s), relevant for the CYP2D6-catalyzed reaction. Further, the CYP2D6 genotype-encoded CYP2D6 activity is solely a measure of the CYP2D6 intrinsic clearance, which means that its impact is modulated by the drugs' hepatic extraction ratio.

Data from several in vitro and in vivo reports argue that the extent of metabolic activity decrease caused by the CYP2D6*10 and CYP2D6*41 alleles is > 50% and in compliance, the recent consensus guidelines decreased the metabolic activity score approximation for CYP2D6*10 from 50% to 25%. The results here obtained, which are based on a very large number of patients, support these data and argue that the decrease in metabolic activity encoded by the CYP2D6Decr alleles is indeed lower than 50% compared with the one of CYP2D6Norm. Related to the CYP2D6*41 allele, the here obtained results are concordant with previously published in vitro and recent in vivo studies with CYP2D6 substrates other than those investigated here (i.e., tamoxifen and voriconazole), which also strongly suggest that the residual metabolic activity encoded by the CYP2D6*41 is very low. Moreover, the differences in metabolic activity in vivo between CYP2D6Nonf/Nonf and CYP2D6Nonf/*41, as well as between CYP2D6Norm/Nonf and CYP2D6Norm/*41, are quite modest. Finally, the results presented in Table 1 argue in favor of the concept that the diplotype activity score can be approximated as the sum of haplotype activity scores with a relatively high level of reliability.

Related to translating the information about CYP2D6 genotype into CYP2D6 metabolizer categories, the results obtained indicate that this concept should be further discussed. In particular, it is clear that there are many distinct diplotypes with the activity scores higher than CYP2D6Nonf/Nonf and lower than the one observed in CYP2D6Norm/Norm, that exhibit very different properties, and that it would be erroneous to place all of them into a single category. One of the solutions would be to divide these diplotypes into two distinct IM categories; the first IM containing CYP2D6Nonf/Decr and CYP2D6Decr/Decr and the second (IM+) containing CYP2D6Norm/Nonf and CYP2D6Norm/Decr. Such a categorization would represent the substantial improvement compared with the existing one; however, it would still not be perfectly precise, because it would ignore the existing differences between distinct CYP2D6 metabolizer categories, the results obtained would still not be perfectly precise, because it would ignore the existing differences between distinct CYP2D6 metabolizer categories, the results obtained would still not be perfectly precise, because it would ignore the existing differences between distinct CYP2D6 metabolizer categories, and it would require additional studies to fully validate the results obtained.

### Limitations

The most important limitation of this report arises from the nature of routine hospital genotyping, which only considered functional variant CYP2D6 alleles common in Europe. Patients were not genotyped for the common CYP2D6*2 allele and consequently the CYP2D6Norm allele comprises both CYP2D6*1 and CYP2D6*2 alleles; however, the CYP2D6*2 allele is postulated to encode normal CYP2D6 metabolic activity and to be close to a CYP2D6*1 equivalent. In addition, rare CYP2D6 alleles were not determined by genotyping and as a result, some patients might be categorized into wrong CYP2D6 genotype-defined subgroups. Considering the high total number of the included patients, however, it is unlikely that subtle differences caused by a handful of miscategorized carriers of rare CYP2D6Nonf alleles affected the overall result directionality and meaning in a significant manner.

## Supporting Information

Supplementary information accompanies this paper on the Clinical Pharmacology & Therapeutics website (www.cpt-journal.com).

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### Table 2 Contributions of CYP2D6Norm, CYP2D6*41, and CYP2D6*9-10, alleles to CYP2D6 diplotype activity score

| Regression coefficient | Risperidone | Venlafaxine | Aripiprazole | All drugs |
|------------------------|-------------|-------------|--------------|-----------|
| Constant               | -102.13 (15.92) | -13.38 (6.87) | 2.33 (8.14) | -3.19 (2.99) |
| \(B_{\text{CYP2D6Norm}}\) | 74.15 (5.10) | 58.23 (1.98) | 44.68 (2.62) | 58.59 (1.81) |
| \(B_{\text{CYP2D6*41}}\) | 10.48 (9.37) | 12.51 (3.87) | 11.82 (4.89) | 10.39 (3.44) |
| \(B_{\text{CYP2D6*9-10}}\) | 23.47 (11.76) | 22.81 (4.29) | 11.56 (5.52) | 19.84 (3.95) |
| Dose                   | 4.15 (0.86) | 1.53 (0.25) | 1.37 (0.31) | N/A        |
| Withdrawal time        | 11.164 (1.90) | -0.16 (0.02) | -1.12 (0.23) | N/A        |

Unstandardized regression coefficients with standard error in parentheses for CYP2D6Norm, CYP2D6*41, and CYP2D6*9-10 alleles, daily dose (mg/day), and time between drug intake and blood sampling (hours).
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CONFLICT OF INTEREST
All authors declared no competing interests for this work.

AUTHOR CONTRIBUTIONS
M.M.J., E.M., and M.I.-S. wrote the manuscript. M.M.J. designed the research. M.M.J. and R.L.S. performed the research. M.M.J. analyzed the data.

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